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Taxonomy of the Genus *Ceratocystis*¹

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The genus *Ceratocystis* contains ascomycetous fungi of considerable economic importance. Several species are pathogenic on crop plants, some cause forest and shade tree diseases, some are associated with stain of forest products, others are known to be associated with insect attacks on wood, and there are a few species to which no particular economic role has been attributed. The genus has received attention from pathologists and mycologists but no comparative morphological study of the entire genus has been attempted. Accordingly, all available type and authentic herbarium material, as well as cultures, have been studied in the preparation of this paper.

MATERIAL AND METHODS

Herbarium material consisted of specimens on wood and other natural substrate or of dried cultures grown on agar media or sterilized wood blocks. To study the material a small bit of the substrate with attached perithecia was removed, wet with 95 percent alcohol, and then mounted in 2 percent KOH solution. The diameter of the base and the length of the neck of the perithecia were measured under a dissecting microscope fitted with an ocular micrometer. All other measurements were made with a microscope x 430 or, in the case of minute objects like ascospores, under oil immersion x 930.

Macroscopic cultural features were described from colonies growing in a Petri plate. For microscopic cultural studies portions of the mycelial mat were removed from different parts of an actively growing colony and mounted in distilled water. Perithecia produced in culture were compared with those found on the natural substrate and differences, if any, were noted.

MORPHOLOGY OF CERATOCYSTIS

Perfect Stage.—In the genus *Ceratocystis* perithecia are characterized by an enlarged base and slender neck, evanescent asci, and hyaline ascospores. The perithecia are produced singly or in clusters, are

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superficial or with the bases immersed in the substrate, and are not formed in or on a stroma.

The pale brown to black base is globose to flask-shaped and 50–600 μ in diameter. The wall of the perithecium, composed of irregularly shaped pseudoparenchymatous cells, is often net-like in appearance. In some species the base of the perithecium is ornamented with short conical spines (Plate 4, fig. 3), or with globose to irregularly shaped protuberances (Plate 4, figs. 1 and 2), or with brown to black, thin- to thick-walled, septate, unbranched hyphal hairs (Plate 4, fig. 4). Ornamentation is usually lacking, however, or is present only as an outgrowth of undifferentiated hyphae.

The neck of the perithecium is dark brown to black except for a pale brown to hyaline apex. It is composed of septate, elongated hyphal cells forming a hollow cylinder through which the ascospores move, exuding to form a slime drop at the tip. Its dimensions are 150–9000 μ in length, 15–120 μ in diameter at the base, and 5–80 μ at the tip. Ornamentation is usually lacking on the neck except at the apex where a whorl of hyaline to pale brown ostiolar hyphae are often formed. Ostiolar hyphae vary in number from 1–2 to more than 50 and in size from 10–300 \times 1–4 μ . Within a given species, variation occurs both in number and size of ostiolar hyphae but they have systematic value when correlated with other features. In addition to ostiolar hyphae one small group of species also has rings or annuli along the sides of the neck.

Because the asci are evanescent they are difficult to find in all stages of perithecial development. The walls of immature asci are very thin or are even lacking in some species as shown by cytological investigations (Andrus, 1936; Andrus and Harter, 1933; Bakshi, 1951b; Elliott, 1925; Gwynn-Vaughan and Broadhead, 1936; Hutchinson, 1950; Mittman, 1932; Moreau, F., 1952; Sartoris, 1927). Any vestige of a wall usually disappears with the development of the ascospores. The ascospores are therefore enveloped in a mass of oily gelatinous material composed of the disintegrated ascus walls and broken down remnants of the cells lining the interior of the perithecial cavity. Hydrostatic pressure (Sartoris, 1927) develops inside the perithecium and the gelatinous mass with imbedded ascospores is forced out at the tip as a white to yellowish droplet.

Mature ascospores are hyaline, one-celled, smooth, always imbedded in a slime sheath, and vary from 1.5–14 μ long by 0.5–6 μ wide. The shape is highly variable. One feature warrants particular attention. In some species, due to the presence of brim-like or flanged appendages, the spores appear hat-shaped (Plate 1, fig. 3) or quadrangular (Plate 1, fig. 1) in outline.

Imperfect Stages.—The species of *Ceratocystis* may be divided into two general categories based on the conidial stages, first those species which produce endoconidia, and secondly those species with only exogenously produced conidia. In both types only one-celled conidia or endoconidia have been noted. Endoconidia develop inside endoconidiophores and usually exude in chains from their tips. In addition to the endoconidia, some species in the first group also form conidia exogenously. (See Plate 2 for endoconidial stages.)

The majority of the species of *Ceratocystis* belong in the second group. Exogenous conidial formation falls into two categories, mycelial conidia and conidia of the *Graphium* or *Leptographium* type.

Mycelial conidia are produced directly on the vegetative mycelium or on slightly differentiated conidiophores. Three general types of formation are recognized. In the first type one to a few conidia develop from short sterigmata at the tips of or in whorls along the sides of the conidiophore (Plate 3, fig. 2). While still attached these first formed or primary conidia often produce secondary conidia, the secondary conidia produce tertiary conidia, and so forth. When mounted for microscopic examination the whole structure usually disintegrates. The conidia are of various sizes and shapes. This method of asexual spore formation most closely resembles that of the form genus *Cladosporium*.

In the second type of mycelial conidial formation the conidia are produced in clusters along the sides of and at the tips of the conidiophores (Plate 3, fig. 3). Since these conidia develop on closely arranged short sterigmata, a conidiophore from which the conidia have fallen presents an irregularly roughened to knobby appearance. The conidia are ovoid to ellipsoid, usually pointed at the attached end, and are more regular in size and shape than the *Cladosporium*-like type. Münch (1907) designated this mode of conidial formation as the "Büschel-form."

In the third type single conidia may be cut off successively at the tip of the conidiophore where the conidia often become imbedded in a small drop of liquid (Plate 3, fig. 5). Sometimes several droplets coalesce and a colony presents a beaded appearance under low magnification. The conidia are all approximately of the same size and are cylindric to ellipsoid. This type of conidial formation most closely resembles that of the form genus *Cephalosporium*.

Intermediate stages of the above three types of mycelial conidial formation are frequently encountered. In *Ceratocystis pilifera* all three types as well as intermediate stages are produced. In *C. minor* a modified "Büschel-form" is produced on a short branched structure which may approach the *Leptographium* type.

In the *Graphium* and the *Leptographium* types conidia are produced on a specialized fruiting structure, a stalk terminated by a head of repeatedly branched conidiophores. The entire head is embedded in a slime drop containing the conidia. In the *Leptographium* type the stalk is a simple, brown, septate, relatively thick-walled hypha consisting of 3-10 cells (Plate 3, fig. 4). In the *Graphium* type it is composed of several to many agglutinated dark hyphae, and the whole structure, the stalk and the ramified head, is called a coremium or a synnema (Plate 3, fig. 1). In both types individual conidia are formed acrogenously at the tips of the conidiophores which make up the head. The conidia are usually ellipsoid to cylindric and uniform in size.

Aino Mathiesen-Käärík (1953) believes that the imperfect name *Leptographium* should be restricted to those species in which the conidia are produced on a single stalk with a ramified head, a concept with which I agree. In many of the species of *Ceratocystis* which have a *Graphium* stage, the simplest fruiting structure may contain only one

stalk and thus resemble a *Leptographium*, but there will always be present numerous coremia of the typical *Graphium* type. Species which produce conidia of the *Leptographium* and the *Graphium* type usually also have one of the mycelial conidial types.

CULTURAL CHARACTERISTICS

All species of *Ceratocystis* can be grown on artificial media. Not all species, however, retain their vitality in culture. After two or three transfers a sterile mycelium often results which is of no value for identification. Freshly isolated cultures are usually distinctive. Useful macroscopic cultural characters are the habit of growth, color changes, rate of growth, and odor, if any.

The habit of growth varies considerably. Colonies may be almost completely appressed with little or no aerial growth as in *Ceratocystis minuta*, or there may be an abundant aerial growth as in *C. adiposa*. In some species, *C. minor* for example, different isolates exhibit various growth habits. The character of the aerial mycelium may vary from delicate and silky to coarse and fibrous, or to densely matted with all possible intermediates.

The color of young colonies is always hyaline or white, and in some species the color remains unchanged. In most species, however, a definite change occurs after a few days, and the older portions of the colonies gradually become darker. The color varies either from pale brown to black or from gray through greenish gray or olivaceous to black. In many species the aerial mycelium remains white and masks the darker mat of submerged hyphae, consequently the undersurface of the agar in a Petri plate often gives a better indication of color change.

The growth rate of a species is the radial advance in millimeters of a colony grown on 2.5 percent malt agar² at 22–24° C. for 10 days. Three general rates are recognized: (1) slow, with radial growth up to 10 mm.; (2) intermediate, from 10–30 mm.; and (3) rapid, over 30 mm.

The odor produced by most species growing in culture is not distinctive. Cultures of many of the endoconidial group smell like banana oil or fruit, and in some of the other species an acidic, or honey-like, or aromatic odor is characteristic.

The conidia and the conidiophores have already been discussed. The microscopic features of the hyphae of all species are so similar that they are of little importance taxonomically. Special hyphal features like sclerotia, globose cells, and serpentine growth are dealt with in the specific descriptions.

SUBSTRATE

Most species of *Ceratocystis* are found on wood. The sapwood is mainly attacked, particularly the rays, which seem to contain the principal source of food.

On standing trees the fungi are usually found in conjunction with

² 2.5% malt agar contained the following: 25 grams of Difco malt extract and 15 grams of Difco agar made up to 1 liter.

insects which are the principal vectors. Many species of *Ceratocystis* have been shown to be intimately associated with certain genera and often definite species of beetles (Bakshi, 1950, 1951a; Grossmann, 1932; Leach, 1940; Mathiesen, 1950, 1951; Mathiesen-Käärrik, 1953; Nisikado and Yamauti, 1933; Rennerfeldt, 1950; Rumbold, 1931, 1936, 1941; Siemaszko, 1939). In some species the fungus is the causal organism in the death of a tree, for example, in the Dutch elm disease (Boyce, 1948); in some a symbiotic relationship exists where the combined effects of the insect attack and the fungus produce death (Rumbold, 1931); in some others the fungus grows in the wood and the insects feed on the spores and the hyphae of the fungus (Leach, 1940); and in some the fungi are associated with the beetle attacks, but apparently contribute nothing to the welfare of the insects (Siemaszko, 1939; Mathiesen, 1951).

A number of species are found on logs and lumber and some of these cause blue stain. Often the same fungi are found on both standing timber and on freshly cut lumber (Davidson, 1935). *Ceratocystis ips*, usually associated with bark beetles in the genus *Ips*, is one of the most common staining fungi in the southeastern United States. Species associated with lumber stain may occur as secondary invaders on standing trees. Species causing stain may be introduced onto freshly cut uncontaminated lumber by wind, rain, and insects, or by contact with contaminated material (Verrall, 1941).

A few species of *Ceratocystis* are found on substrates other than wood. *C. narcissi* occurs on the scales of narcissus bulbs; *C. fimbriata*, causing a destructive sweet potato black rot, is also the causal organism associated with cankers on coffee, rubber, and plane trees. Other substrates are sugar cane, coconut and date palms, pineapple, and cacao.

TAXONOMIC HISTORY OF CERATOCYSTIS

Ceratocystis (apparently from *ceratos* = horn and *cyst* = pouch or sac) was established by Ellis and Halsted (in Halsted, 1890). No formal generic description was given in the original paper or in any other publication so far as can be ascertained. *Ceratocystis* was described as a monotypic genus, and an excellent specific description of *C. fimbriata*, the type species of the genus, was given by Halsted and Fairchild (1891), in which the generic concept was also clearly established. In the original description the perithecia and the ascospores produced in evanescent asci were misinterpreted as pycnidia and conidia. The subsequent history of the type species is very much confused. On the basis of the supposed pycnidial stage Saccardo (1892) discarded *Ceratocystis* and transferred the species to *Sphaeronaema*. Elliott (1923) established that the "pycnidia" of *S. fimbriatum* were perithecia and he transferred the species to *Ceratostomella*. Subsequently the species was transferred to *Ophiostoma* by Nannfeldt (in Melin and Nannfeldt, 1934) and to *Endoconidiophora* by Davidson (1935). The generic name *Ceratocystis* was revived by Bakshi (1950) and applied to the group of species including *C. fimbriata* and its relatives. No emended description for *Ceratocystis* has been found in the literature.

The following genera are placed in synonymy or are excluded:

1. *Sphaeria* Haller ex Fries (in part), Syst. Myc. 2: 319. 1822.
2. *Ceratostoma* Fuckel (1869), not *Ceratostoma* Fries, Obs. 2: 317. 1818, and Summa Veg. Scand., p. 396. 1849. Excluded. The genus as now defined has brown ascospores in persistent asci.
3. *Ceratostomella* Saccardo emend. von Höhnelt (1918). Excluded. The species retained in *Ceratostomella* in the sense of von Höhnelt have hyaline ascospores formed in asci which persist until spore discharge. Von Arx (1952), who studied the type of *Ceratostomella* Sacc., concurs in the concept established by von Höhnelt.
4. *Sphaeronaemella* Karsten emend. Seeler (1943). Excluded. This monotypic genus has hyaline ascospores produced in evanescent asci. It is here recognized as a separate genus having orange to yellow perithecia on rotten discomycetes.
5. *Rostrella* Zimmermann (1900). A homonym of *Rostrella* Fabre (1878).
6. *Endoconidiophora* Münch (1907). A monotypic genus when described; it is considered here a synonym of *Ceratocystis*.
7. *Linostoma* von Höhnelt (1918). A homonym of *Linostoma* Wallich (1831) in the Thymelaeaceae (Angiospermae).
8. *Ophiostoma* H. & P. Sydow (1919). *Ophiostoma* was proposed for *Linostoma* von Höhnelt. It is considered here as a later synonym for *Ceratocystis*.
9. *Grosmannia* G. Goidanich (1936a). A genus proposed to include species of *Ophiostoma* which have *Leptographium* as the imperfect stage. It is considered here a synonym of *Ceratocystis*.
10. *Fugascus* Falck (1947). A nomen nudum.

A new name, *Ceratocystis perparvispora* (p. 46) is proposed for *Ceratostomella* (*Ophiostoma*) *microspora* Davids.

The following species are placed in synonymy for the first time:

- Ceratocystis longirostellata* = *Ceratocystis capillifera*
Ceratostomella exigua = *C. minor*
Ceratostomella pini = *C. minor*
Ceratostomella pseudotsugae = *C. minor*
Ceratostomella querci = *C. piceae*
Endoconidiophora bunae = *C. moniliformis*
Endoconidiophora virescens = *C. coeruleascens*

New combinations are made in the following:

- | | |
|---------------------------------------|--|
| <i>Ceratocystis albida</i> (Math.-K.) | <i>C. montia</i> (Rumb.) |
| <i>C. brunneo-ciliata</i> (Math.-K.) | <i>C. multiannulata</i> (Hedgc. & Davids.) |
| <i>C. clavata</i> (Math.) | <i>C. narcissi</i> (Limber) |
| <i>C. fagacearum</i> (Bretz) | <i>C. obscura</i> (Davids.) |
| <i>C. floccosa</i> (Math.) | <i>C. olivacea</i> (Math.) |
| <i>C. leptographioides</i> (Davids.) | <i>C. rostrocyllindrica</i> (Davids.) |
| <i>C. minor</i> (Hedgc.) | <i>C. tetropii</i> (Math.) |
| <i>C. minuta</i> (Siem.) | |

In this paper 63 species are considered of which 39 are recognized as valid, 7 are placed in synonymy for the first time, and 13 are species

imperfectly known, or species for which no material could be obtained for study, or are excluded (see page 55).

DISTRIBUTION OF SPECIES

Study notes have been placed in the packets of all material studied, which served as the basis for the distribution records. The herbaria are indicated by the abbreviations recommended by Lanjouw and Stafleu (1952). When the distribution is taken from the literature, the author and date are given. Many citations are taken from the Review of Applied Mycology, hereafter referred to as R.A.M. Additional abbreviations are AM-K, Aino Mathiesen-Käärik, Experimentalfältet, Sweden; CBS, Centraal bureau voor Schimmelcultures, Baarn, Netherlands; FPRL-C, Forest Products Research Laboratory, Ottawa, Canada; FPRL-E, Forest Products Research Laboratory, Princes Risborough, England; SYRF, State University of New York College of Forestry, Syracuse, New York.

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THE GENUS CERATOCYSTIS

Ceratocystis belongs in the Plectascales as defined by Nannfeldt (1932, and in Melin and Nannfeldt, 1934), based on the irregular arrangement of the asci in the perithecium. Inasmuch as no generic diagnosis was given either in the original description or subsequently when the genus was transferred to the Ascomycetes, the following generic diagnosis is given:

CERATOCYSTIS Ellis and Halsted, emend. Bakshi Commonw. Mycol. Instit., Mycol. Paper 35: 2. 1951.

N. J. Agr. Expt. Sta. Bul. 76: 14. 1890, and Jour. Mycol. 7: 1. 1891.

Perithecia produced singly or in clusters, nonstromatic, the bases brown to black with elongate black necks; perithecial centrum plectascaeous; paraphyses lacking; asci evanescent, with 8 spores; ascospores hyaline, 1-celled, exuded from the ostiole in a sticky matrix. Imperfect stages include endogenously and exogenously produced conidia.

KEY TO THE SPECIES

1. Ascospores with a gelatinous sheath which often forms brim-like or flanged appendages, hat-shaped to quadrangular in outline (Plate 1, Figs. 1-5)
 2. Ascospores with a thick, almost transparent sheath, cylindrical to ellipsoid or a few hat-shaped
 3. Ostiolar hyphae absent
 4. Ascospores 4-6 μ long
 5. Ascospores 3-4 μ wide; necks rarely exceeding 500 μ in length.....11. *C. penicillata*
 5. Ascospores 2-3 μ wide; necks up to 900 μ long.....12. *C. piceaperda*
 4. Ascospores 2.5-4 μ long.....14. *C. rostrocylicindrica*
 3. Ostiolar hyphae present.....15. *C. polonica*
 2. Ascospores with sheath modified into flanged appendages, quadrangular in outline
 6. Ostiolar hyphae present
 7. Necks more than 500 μ long; ostiolar hyphae pale brown and shaped like a corkscrew.....20. *C. brunneo-ciliata*
 7. Necks less than 500 μ long; ostiolar hyphae straight
 8. Ostiolar hyphae hyaline; ascospores rectangular in outline; imperfect stage a *Leptographium*.....16. *C. leptographioides*
 8. Ostiolar hyphae pale brown; ascospores of various quadrangular shapes due to two unsymmetrically placed brims; imperfect stage a *Graphium*
 9. Ascospores 5-6.5 x 3-4 μ ; ostiolar hyphae 40-80 μ long.....17. *C. olivacea*
 9. Ascospores 4-5.5 x 2-2.5 μ ; ostiolar hyphae up to 40 μ long.....18. *C. obscura*
 6. Ostiolar hyphae absent
 10. Diameter of the base rarely exceeding 200 μ ; imperfect stage a *Graphium*.....19. *C. ips*
 10. Diameter of the base up to 440 μ ; mycelial conidia only present.....34. *C. montia*
 2. Ascospores with sheath forming one or sometimes two brims, hat-shaped
 11. Ascospores often with two brims; ostiolar hyphae pale brown compare.....17. *C. olivacea* and 18. *C. obscura*
 11. Ascospores with one brim; ostiolar hyphae, if present, hyaline
 12. Ostiolar hyphae present
 13. Base of the peritheciium ornamented with conical spines.....5. *C. moniliformis*
 13. Base of the peritheciium unornamented or with undifferentiated hyphae
 14. Diameter of the base 250-450 μ ; imperfect stage a *Leptographium*.....15. *C. polonica*
 14. Diameter of the base not exceeding 250 μ ; imperfect stage endoconidial
 15. Ostiolar hyphae 15-20 in number, 20-35 μ long...4. *C. variospora*
 15. Ostiolar hyphae 8-15 in number, 50-90 μ long...3. *C. fimbriata*
 12. Ostiolar hyphae absent
 16. Imperfect stage a *Leptographium*; growth in culture rapid compare.....11. *C. penicillata* and 12. *C. piceaperda*
 16. Imperfect stage *Graphium*-like; growth in culture slow.....21. *C. galeiformis*
1. Ascospores without thick gelatinous sheat or appendages, of various shapes, filiform, cylindrical, ellipsoid, kidney-shaped, halfmoon-shaped, or crescent-shaped (Plate 1, Figs. 6-14)
 17. Ascospores mostly 6 μ long or longer, filiform, elongate-ellipsoid, halfmoon-shaped, or kidney-shaped
 18. Ascospores filiform, 8-13 x 0.5-1.5 μ38. *C. minuta*
 18. Ascospores halfmoon-shaped, more than 3.5 μ thick
 19. Ostiolar hyphae 150-300 μ long; base of the peritheciium up to 600 μ in diameter.....2. *C. major*
 19. Ostiolar hyphae up to 130 μ long; base of the peritheciium up to 350 μ in diameter.....1. *C. adiposa*

18. Ascospores elongate-ellipsoid or kidney-shaped, not exceeding $3.5\ \mu$ thick
20. Perithecia with irregularly shaped appendages
 21. Ostiolar hyphae blunt at the tips, up to $65\ \mu$ long...7. *C. radicola*
 21. Ostiolar hyphae pointed, up to $150\ \mu$ long.....6. *C. paradoxa*
20. Perithecia with long, dark, hyphal hairs, or with undifferentiated hyphae, or unornamented
 22. Perithecia ornamented with long dark brown, straight, septate, unbranched hyphal hairs.....9. *C. coerulescens*
 22. Perithecia unornamented or with undifferentiated hyphae
 23. Necks short, stout; ostiolar hyphae average $75\ \mu$ long.....8. *C. fagacearum*
 23. Necks long, slender; ostiolar hyphae average $22\ \mu$ long. 23. *C. cana*
17. Ascospores mostly shorter than $6\ \mu$, short ellipsoid, cylindrical, bean-shaped, kidney-shaped, or crescent-shaped
24. Ascospores not curved, cylindrical to short-ellipsoid
25. Ascospores cylindrical; imperfect stage endoconidial.....10. *C. autographa*
25. Ascospores ellipsoid; conidia produced exogenously
 26. Ascospores $3.3\text{--}4.8\ \mu$ long; imperfect stage a *Leptographium*.....13. *C. serpens*
 26. Ascospores not over $3.5\ \mu$ long; imperfect stage a *Graphium* or mycelial conidia
 27. Necks over $500\ \mu$ long; on wood
 28. Ostiolar hyphae lacking; mycelial conidia only present.....35. *C. perparvispora*
 28. Ostiolar hyphae brown, spirally coiled; imperfect stage *Graphium*-like.....25. *C. clavata*
 27. Necks less than $400\ \mu$ long; on narcissus bulbs....39. *C. narcissi*
24. Ascospores curved, bean-shaped, kidney-shaped, crescent-shaped, like sections of an orange, or short cylindrical
 29. Necks not exceeding $400\ \mu$ in length
 30. Ascospores bean-shaped, $3.5\text{--}4\ \mu$ long.....37. *C. albidia*
 30. Ascospores crescent-shaped, or shaped like sections of an orange, somewhat tapered at the ends, $4\text{--}6\ \mu$ long
 31. Necks $180\text{--}360\ \mu$ long; ostiolar hyphae numerous, up to $30\ \mu$ long; imperfect stage a *Graphium*.....26. *C. ulmi*
 31. Necks up to $150\ \mu$ long; ostiolar hyphae $6\text{--}10$ in number, up to $19\ \mu$ long mycelial conidia only present.....36. *C. minor*
 29. Necks over $400\ \mu$ long, often several mm. in length
 32. Ostiolar hyphae absent
 33. Necks $25\text{--}40\ \mu$ in diameter at the tip; ascospores ellipsoid to curved, some almost crescent-shaped, $2.5\text{--}4 \times 1\text{--}1.5\ \mu$; imperfect stage a *Leptographium*.....14. *C. rostracylindrica*
 33. Necks $11\text{--}16\ \mu$ in diameter at the tip; ascospores bean-shaped, $3\text{--}5 \times 1.5\text{--}2\ \mu$; mycelial conidia only present....32. *C. ambrosia*
 32. Ostiolar hyphae present
 34. Necks regularly with several annuli in addition to the terminal fringe of ostiolar hyphae, often several mm. long
 35. Ostiolar hyphae up to $60\ \mu$ long; bases unornamented.....27. *C. pluriannulata*
 35. Ostiolar hyphae up to $12\ \mu$ long; bases with globular protuberances.....28. *C. multiannulata*
 34. Necks rarely with additional annuli; rarely over 2 mm. long
 36. Ostiolar hyphae usually much more than $30\ \mu$ long
 37. Ostiolar hyphae pale brown, spirally coiled; imperfect stage *Graphium*-like.....25. *C. clavata*
 37. Ostiolar hyphae hyaline, at least at the tips, straight; mycelial conidia only present
 38. Ostiolar hyphae erect, sharply pointed, often pale brown at the base, hyaline at the tip; ascospores short cylindrical, curved.....33. *C. tetropii*
 38. Ostiolar hyphae spreading to form a crown; ascospores bean-shaped to kidney-shaped

- 39. Ostiolar hyphae slender and attenuated at the tips, up to 100 μ long.....30. *C. capillifera*
- 39. Ostiolar hyphae not tapered, blunt at the tips, up to 60 μ long.....29. *C. pilifera*
- 36. Ostiolar hyphae usually less than 30 μ long
- 40. Ascospores 5-6(-6.5) μ long; conidia subglobose to globose.....23. *C. cana*
- 40. Ascospores mostly less than 5 μ long; conidia of other shapes
- 41. Ostiolar hyphae not exceeding 15 μ in length
- 42. Mycelial conidia only present.....31. *C. schrenkiana*
- 42. Imperfect stage a *Graphium*.....24. *C. floccosa*
- 41. Ostiolar hyphae 15-25 μ in length
- 43. Imperfect stage a *Graphium*; cultures with abundant aerial mycelium.....22. *C. piceae*
- 43. Mycelial conidia only; cultures with little or no aerial mycelium.....32. *C. ambrosia*

Section 1. Species with an endoconidial imperfect stage (Endoconidial Group) (For species with *Graphium* or *Leptographium* imperfect stages see No. 11 et seq. and for those with mycelial conidial stages see No. 27 et seq.)

I. Spores halfmoon-shaped or hat-shaped

A. Ascospores halfmoon-shaped

1. CERATOCYSTIS ADIPOSA (Butl.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Sphaeronaema adiposum Butl., India Dept. Agr. Mem., Bot. Ser. 1(3): 40. 1906.

Ceratostomella adiposa (Butl.) Sart., Jour. Agr. Res. 35: 585. 1927.

Ophiostoma adiposum (Butl.) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408. 1934.

Endoconidiophora adiposa (Butl.) Davids., Jour. Agr. Res. 50: 802. 1935.

Perithecia superficial, the bases black, globose, 230-350 μ in diameter, ornamented with short, dark brown, thick-walled hyphae 20-25 x 3-6 μ , or with undifferentiated hyphae; necks long, black, slender, tapered slightly, up to 3000 μ long, 40-50 μ in diameter at the base and 20-30 μ at the tip; ostiolar hyphae numerous, spreading, sometimes branching, pale brown, hyaline at the tips, not tapered, septate, thin-walled, up to 150 x 3 μ ; asci not seen; ascospores halfmoon-shaped concave to plane on one side and convex on the other, pointed at the ends, 6-7.5 x 3.5-5 μ (Plate 1, fig. 12).

Cultures hyaline at first, quickly turning dark green to black, with abundant aerial mycelium forming a stringy coarse mat which turns gray then black in 6-9 days; undersurface black; endoconidia produced in 3 days; perithecia appearing in 2-5 days, maturing in 7-9 days; growth rapid, 45 mm.; odor not distinctive or of banana oil. Aerial hyphae at first hyaline, quickly becoming pale brown to almost black, frequently branched, thin-walled, frequently septate, forming a loosely interwoven black mat above the surface of the agar, 2.5-8 μ in diameter; submerged hyphae similar except up to 12 μ in diameter. Endoconidiophores pale brown, unbranched, straight, septate near the base, up to 30 x 4.5 μ (Plate 2, fig. 3); endoconidia extremely variable, borne at the tip of the endoconidiophores in chains, hyaline to pale brown at first, remaining pale and thin-walled or becoming black and thick-walled, smooth

or covered with papillae arranged in indistinct rows, ellipsoid to subglobose, $9-19 \times 5-19 \mu$ or " $9-25 \times 4.5-18 \mu$ " (Butler).

Illustrations: Butler, E. J., 1906. India Dept. Agr. Mem., Bot. Ser. 1(3): 35-40, Plate X, figs. 47-58, Plate XI, figs. 59-61; Sartoris, G. B., 1927. Jour. Agr. Res. 35: 577-585, Figs. 1-4; Davidson, R. W., 1935. Jour. Agr. Res. 50: 789-807, Fig. 3A-F.

This species was first described from sugar cane culms in India (Butler, 1906; also IMI) and has been recorded on various substrates from Malaya (R.A.M. 12: 355), China (BPI), Mexico (BPI), Louisiana (Sartoris, 1927), and Maryland (BPI, FH, NY).

C. adiposa and the next species, *C. major*, are distinct from other species of *Ceratocystis* in having halfmoon-shaped ascospores, large

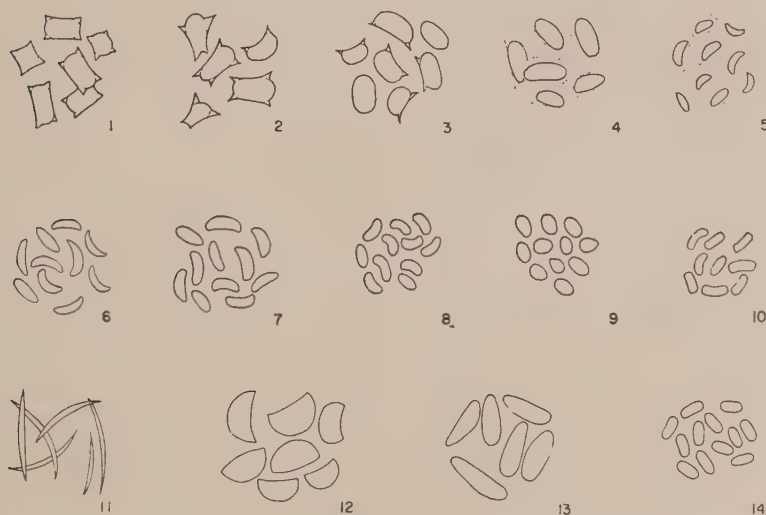


PLATE 1.—FIGS. 1-14.—Ascospores of species of *Ceratocystis*. All $\times 750$. FIG. 1. Quadrangular spores of *C. ips*. FIG. 2. Unsymmetrically quadrangular spores of *C. obscura*. FIG. 3. Hat-shaped spores of *C. fimbriata*. FIG. 4. Spores of *C. penicillata* with a thick gelatinous sheath or sometimes hat-shaped. FIG. 5. Spores of *C. rostracylindrica* with a thick gelatinous sheath. FIG. 6. Crescent- or sickle-shaped spores of *C. minor*. FIG. 7. Spores of *C. pilifera* bean-shaped or shaped like sections of an orange. FIG. 8. Spores of *C. multiannulata* usually bean-shaped. FIG. 9. Broadly ellipsoid spores of *C. narcissi*. FIG. 10. Spores of *C. tetropii* short cylindrical, slightly curved, blunt at the ends. FIG. 11. Filiform spores of *C. minuta*. FIG. 12. Halfmoon-shaped spores of *C. adiposa*. FIG. 13. Elongate-ellipsoid spores of *C. radiculicola*. FIG. 14. Cylindrical spores of *C. autographa*.

perithecia with long necks, and long, pale brown ostiolar hyphae. The two species are very similar but may be separated on the basis of the longer ostiolar hyphae, larger perithecial bases, and longer ascospores in *C. major*. Further work on the two species may show that *C. major* is a synonym of *C. adiposa*.

2. CERATOCYSTIS MAJOR (van Beyma) C. Moreau, *Revue de Mycologie, Suppl. Colonial* 17: 22. 1952.

Ceratostomella major van Beyma, *Zentralbl. Bakt. Parasit. Infektionskr. Abt. 2*, 91: 348. 1935

Ophiostoma majus (van Beyma) Goid., *R. Staz. Pat. Veg. Bol., Rome, n.s.*, 15: 158. 1935.

Perithecia superficial to partially immersed, the bases black, globose, 300–600 μ in diameter, ornamented with many undifferentiated hyphae on all sides; necks long, slender, black, pale brown at the apex, 1500–5000 μ long, 48–96 μ in diameter at the base and 18–30 μ at the tip; ostiolar hyphae numerous, spreading, pale brown, not tapered, septate, thin-walled, "oft gabelformig verzweigt" (van Beyma), up to 300 x 6 μ ; asci not seen; ascospores halfmoon-shaped, pointed at the ends, 7–8.5 x 3.5–5.5 μ .

Cultures hyaline to grayish white at first, with abundant cottony aerial mycelium, remaining light colored or becoming olive-green to black in 5–8 days; undersurface unchanged or sometimes greenish black; growth rapid, 45 mm.; odor fruitlike. Hyphae hyaline at first, some becoming pale brown, branched, thin-walled, septate, 2–9 μ in diameter. Endoconidiophores at first hyaline then becoming brown, septate near the base, up to 55 x 6 μ ; first formed endoconidia in chains, at first hyaline, becoming pale brown, smooth to slightly rough-walled, cylindrical to subglobose, 10–18 x 6–9 μ ; second type of endoconidia formed in older colonies, from same endoconidiophores as the first type, dark brown to black, rough-walled with spinose projections arranged in longitudinal rows, subglobose, 18–27 x 16–20 μ . *Graphium* stage not seen (from Moreau, C., 1952, p. 20) the coremia 200–650 x 12–25 μ , with stalks dark at the base, clearer at the apex, arising from a small plectenchyma-like structure, composed of 10–20 united, septate, filaments, the cells of the filaments 10–20 x 2–3 μ at the base of the coremia, ramified and smaller at the apex, each filament terminated with a hyaline (gray-brown in mass) spore 2–3 x 1 μ . *Graphium* stage persisting a few weeks in culture, the endoconidial stage becoming dominant.

Illustrations: van Beyma thoe Kingma, F. H., 1935. *Zentralbl. Bakt. Parasit. Infektionskr. Abt. 2*, 91: 345–348, Abb. 1 and 2; Moreau, C., 1952. *Revue de Mycologie, Suppl. Colonial* 17: 17–25, Fig. 1.

This species was first isolated from the air in the Netherlands (van Beyma, 1935; type culture JH-12 from CBS). It has also been found on wood in England (Hutchinson, 1939, 1950) and Lebanon (from "Liban," Moreau, C., 1952: culture JH-68 from CBS).

C. major and the preceding species, *C. adiposa*, are distinct from other species of *Ceratocystis* in having halfmoon-shaped ascospores, large perithecia with long necks, and long, pale brown ostiolar hyphae. The perithecia of *C. major* are much larger than those of *C. adiposa*, and the ostiolar hyphae and ascospores are longer. In culture *C. major* produces a more cottony-appearing colony and the color is less intense. The endoconidia are the same in both species, but in *C. major* less of the darker endoconidia are produced. *C. major* also produces a *Graphium* stage as reported by Moreau, C. (1952), a feature not noted for *C. adiposa*. The *Graphium* stage is also characteristic of some of the species of the *Ophiostoma* group.

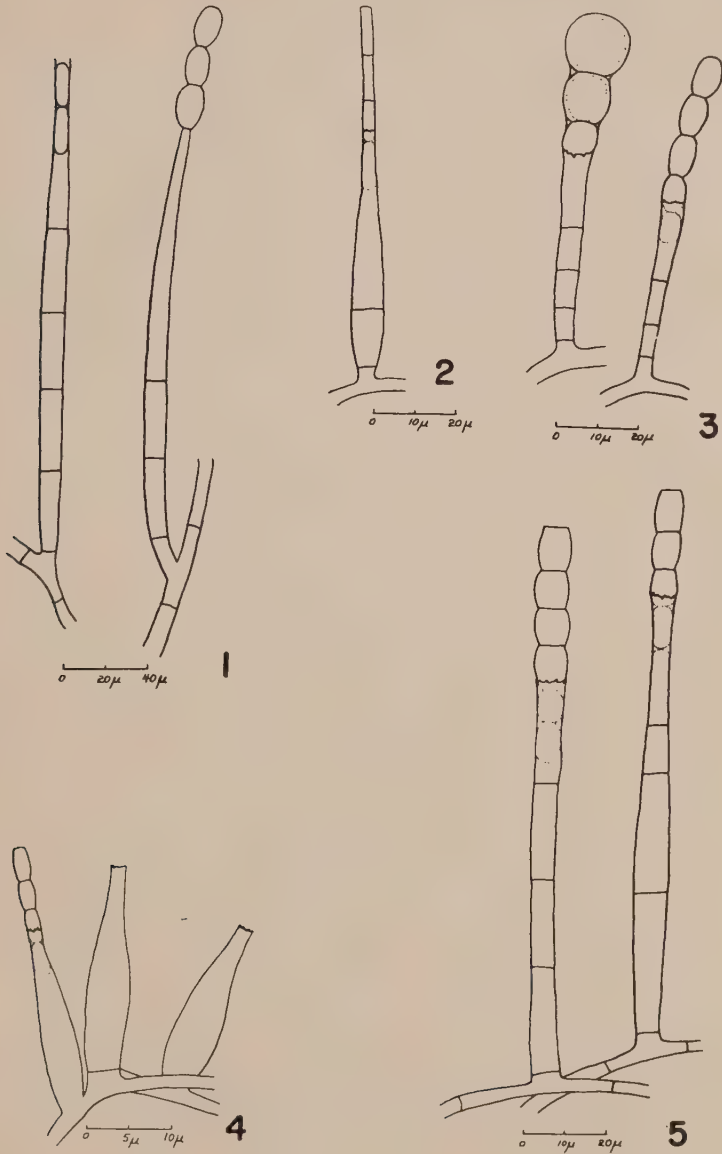


PLATE 2.—FIGS. 1-5.—Endoconidial stages. FIG. 1. *C. paradoxa*. FIG. 2. *C. fimbriata*. FIG. 3. *C. adiposa*. FIG. 4. *C. autographa*. FIG. 5. *C. moniliformis*.

AA. Ascospores hat-shaped

3. CERATOCYSTIS FIMBRIATA Ell. & Halst., N. J. Agr. Expt. Sta. Bul. 76: 14. 1890 and Jour. Mycol. 7: 1. 1891.

Sphaeronaema fimbriatum (Ell. & Halst.) Sacc., Syll. Fung. 10: 215. 1892.

Ceratostomella fimbriata (Ell. & Halst.) Elliott, Phytopath. 13: 56. 1923 (abstract).

Ophiostoma fimbriatum (Ell. & Halst.) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408. 1934.

Endoconidiophora fimbriata (Ell. & Halst.) Davids., Jour. Agr. Res. 50: 800. 1935.

Rostrella coffeae Zimm., Buitenzorg L'Institut Bot. Bul. 4: 19. 1900 and Meded. uit 's Lands Plant. 37: 24. 1900.

Perithecia superficial to immersed, the bases brown to black, globose, sometimes flattened, 130–200 μ in diameter, unornamented or with undifferentiated hyphae attached; necks black, hyaline at the tip, slender, up to 800 μ long, 20–35 μ in diameter at the base and 10–20 μ at the tip; ostiolar hyphae hyaline, slender, tapered to a blunt tip, 8–15 in number, 50–90 x 2–3 μ ; asci not seen; ascospores with gelatinous sheath often forming a brim, hat-shaped, 4.5–8 x 2.5–5.5 μ (Plate 1, Fig. 3).

Cultures hyaline at first with a loose cottony appearance, becoming light brown in 2–3 days, finally turning greenish brown; undersurface of the agar turning dark; conidia produced within 48 hours, and mature perithecia within one week; growth intermediate, 20 mm.; odor of banana oil or fruitlike. Aerial hyphae hyaline to pale brown, branched, thin-walled, septate, the ends nearly all terminating as endoconidiophores, 2–6 μ in diameter; submerged hyphae similar except darker and much interwoven. Endoconidiophores pale brown, hyaline at the tip, septate, thin-walled, tapered toward the tip, 25–125 x 4–6 μ (Plate 2, Fig. 2); endoconidia of two types, one hyaline, cylindrical, truncate at the ends, 11–16 x 4–5 μ , or “16–30 by 4–9 μ ” (Halsted and Fairchild), and the other pale brown to olive-brown, barrel-shaped to subglobose, smooth to rough-walled, 9–16 x 6–13 μ , or “12–19 μ ” long (Halsted and Fairchild).

Illustrations: Halsted, B. D. 1890. N. J. Agr. Expt. Sta. Bul. 76: 7–14, Figs. 3–10; Halsted, B. D., and D. G. Fairchild. 1891. Jour. Mycol. 7: 1–11, Plates II and III; Elliott, J. A. 1925. Phytopath. 15: 417–422, Plates XV and XVI; Harter, L. I., and J. L. Weimer. 1929. U.S.D.A. Tech. Bul. 99, Plates 3 and 6; Andrus, C. F., and L. I. Harter. 1933. Jour. Agr. Res. 46: 1059–1078, Plates 1 and 2, and Figs. 1–6; Davidson, R. W. 1935. Jour. Agr. Res. 50: 789–807, Fig. 2, E–H; Gwynn-Vaughan, H. C. I., and Q. E. Broadhead. 1936. Ann. Bot., n.s. 1: 747–758, Figs. 1–15, and Plates XIII and XIV.

This fungus was first described from sweet potato in New Jersey (Halsted, 1890; TYPE, BPI). It has subsequently been found widespread through the tropics and temperate zones on a variety of substrates. For its distribution see Imperial Mycological Institute, Distribution Maps of Plant Diseases, No. 91. 1945. The form on *Coffea* was described as *Rostrella coffeae*, new genus and species, by Zimmermann (1900). It was studied by von Arx (1952) who transferred it to *Ophiostoma*. Bakshi (1951a) regards it as a synonym of *C. fimbriata*. Another form described as *Endoconidiophora fimbriata* f. *platani* Walter *et al.* (1952) is the cause of a canker disease of plane

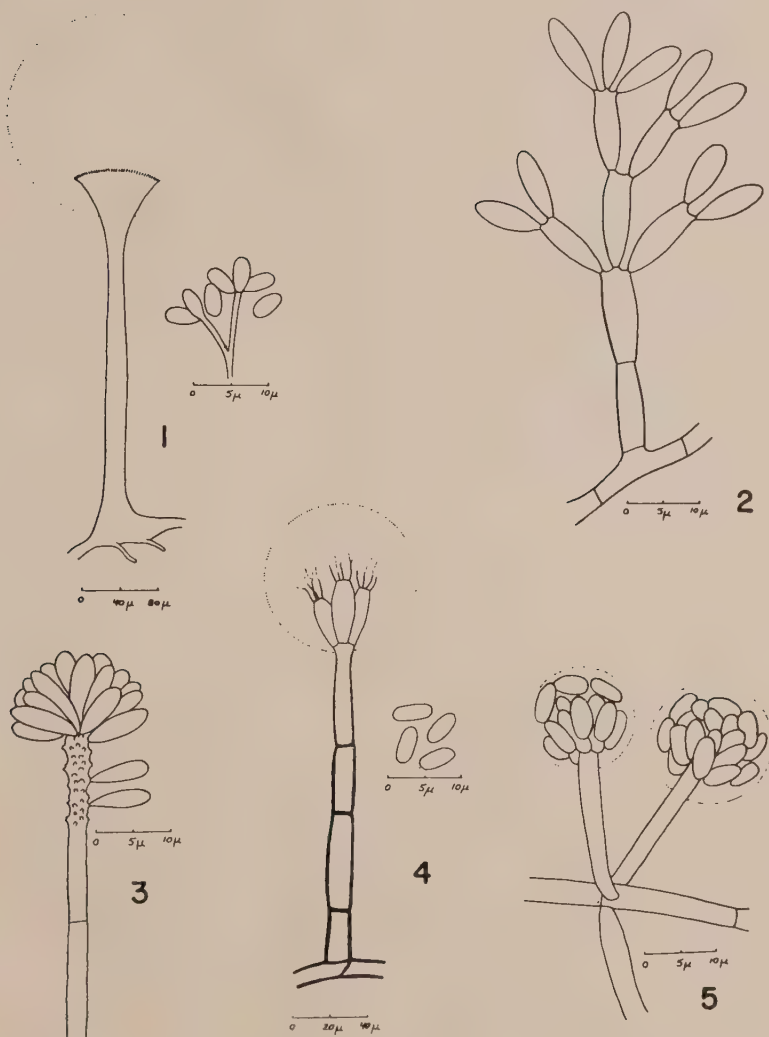


PLATE 3.—FIGS. 1-5.—Conidial stages. FIG. 1. Gross shape and detail of coremium of *C. piceae*. FIG. 2. Cladosporium-like conidial formation of *C. pluriannulata*. FIG. 3. "Büschel-form" of *C. pilifera*. FIG. 4. *Leptographium* stage of *C. serpens*. FIG. 5. Cephalosporium-like conidial formation of *C. telopii*.

trees in the eastern United States. In addition to the type, specimens have been studied on sweet potato (BPI, NY), *Coloesia esculenta* from China (BPI), *Xanthosoma* sp. from the Dominican Republic (BPI), *Hevea* from Malaya (NY), *Coffea* from South America (cultures JH-35 from CBS and JH-55 from J. A. von Arx), and hardwood from the United States (BPI, culture JH-40, CBS).

The species together with *C. variospora* and *C. moniliformis*, the following two species, constitute the endoconidial group with hat-shaped ascospores. *C. moniliformis* has conical spines on the base of the perithecium, and *C. variospora* has shorter ostiolar hyphae, slightly smaller ascospores, and light colored perithecial bases.

4. CERATOCYSTIS VARIOSPORA (Davids.) C. Moreau, *Revue de Mycologie, Suppl. Colonial* 17: 22. 1952.

Endoconidiophora variospora Davids., *Mycologia* 36: 303. 1944.

Ophiostoma variosporum (Davids.) von Arx, *Antonie van Leeuwenhoek* 18: 212. 1952.

Perithecia superficial to partially immersed in the bark, the bases light brown with the area adjacent to the neck black, globose, sometimes flattened, 120–220 μ in diameter, or “150–250 μ ” (Davidson), unornamented or covered with undifferentiated hyphae, some bearing endoconidia; necks black with a hyaline apex, up to 1200 μ long, 30–40 μ in diameter at the base and 10–15 μ at the tip; ostiolar hyphae hyaline, pointed, erect, 10–20 in number, 20–35 x 1–2 μ ; asci not seen; ascospores with gelatinous sheath forming a brim, hat-shaped, 4–6 x 2.5–3.5 μ .

“Cultures white, often developing gray to brown patches... perithecia few or absent; 15 mm. radial growth in 5 days; odor similar to banana oil” (Davidson). Aerial hyphae and submerged hyphae hyaline to pale brown, branched, thin-walled, frequently septate into short cells, 2.5–7.5 μ in diameter. Endoconidiophores of two sizes, the one hyaline to light brown, septate, tapered toward the tip, 50–100 x 2–4 μ , with endoconidia hyaline, cylindrical, truncate, 6–18 x 2–4.5 μ ; the other brown, short, blunt, not tapered, sometimes expanded at the tip, 10–30 x 4–6 μ , with endoconidia hyaline to brown, barrel-shaped to globose, 5–9 x 4.5–7.5 μ . Conidiophores short, hyaline to pale brown side branches, often sympodially arranged, thin-walled, septate, up to 20 x 5 μ with conidia borne singly at the tips, brown, ovoid, smooth, 11–16 x 8–11 μ .

Illustrations: Davidson, R. W. 1944. *Mycologia* 36: 300–306, Fig. 2, A-E.

This fungus is known only from the type collection (TYPE, BPI-FP 94257) on the cambium side of oak bark. It is not important either as a staining organism or as a pathogen on oak.

This species is very similar to the preceding species, *C. fimbriata*, and to the next species, *C. moniliformis*. The latter has conical spines on the base of the perithecium, and the former has longer ostiolar hyphae, slightly larger ascospores, and dark colored perithecial bases. The imperfect stage also varies from that associated with both *C. fimbriata* and *C. moniliformis* as the dark colored conidia are produced singly on alternately branched conidiophores. In this respect *C.*



PLATE 4.—FIGS. 1-4.—Perithecial bases of species of *Ceratocystis*. FIG. 1. Portion of base of *C. radicola* with appendages with irregularly shaped tips. x18. FIG. 2. Base of *C. multiannulata* with black globular protuberances. x16. FIG. 3. Base of *C. moniliformis* with short conical spines. x18. FIG. 4. Base of *C. coerulescens* with long, slender, dark-colored, thin-walled hyphae. x15.

varispora resembles No. 7, *C. radicola*, from which it is easily separated on the basis of the ascospores which are elongate-ellipsoid in *C. radicola*.

5. *CERATOCYSTIS MONILIFORMIS* (Hedgc.) C. Moreau, *Revue de Mycologie*, Suppl. Colonial **17**: 22. 1952.

Ceratostomella moniliformis Hedgc., *Mo. Bot. Gard. Ann. Rpt.* **17**: 78. 1906.

Ophiostoma moniliforme (Hedgc.) H. & P. Sydow, *Ann. Mycol.* **17**: 43. 1919.

Ophiostoma moniliforme (Hedgc.) H. & P. Sydow, *Ann. Mycol.* **17**: 43. 1919.

Endoconidiophora moniliformis (Hedgc.) Davids., *Jour. Agr. Res.* **50**: 800. 1935.

Endoconidiophora bunae Kitajima, *Imp. Forestry Expt. Sta. Meguro, Tokyo, Bul.* **35**: 126. 1936.

Ceratocystis wilsoni Bakshi, *Commonw. Mycol. Inst., Mycol. Paper* **35**: 5. 1951.

Perithecia superficial, the bases black, appearing brown under the microscope, globose to pear-shaped, 120–160 μ in diameter, up to 250 μ in vertical dimension when pear-shaped, ornamented with brown, short, conical spines, thick- to thin-walled, occasionally septate, tapered to a point, often bulbous at the base, up to 30 x 8 μ (Plate 4, Fig. 3.); necks black, up to 900 μ long, 20–30 μ in diameter at the base and 10–15 μ at the tip; ostiolar hyphae 8–10 in number, hyaline, tapered to a point, up to 25 x 1.5 μ ; asci not seen; ascospores with gelatinous sheath forming a brim, hat-shaped, 3–5.5 x 2–2.5 μ .

Cultures white at first, turning gray to brown in 3–5 days; perithecia appearing in 3–5 days, maturing in one week; undersurface of the agar remaining unchanged; growth rapid, 45 mm.; odor of banana oil. Hyphae pale brown, branched, septate, thin-walled, 2–8 μ in diameter. Endoconidia of two kinds, one long, often septate, enlarged at the base, tapered to the tip, up to 60 μ tall, 3.5–5.5 μ in diameter at the base and 2–3 μ at the tip, with endoconidia hyaline, cylindrical, truncate, smooth, often in short chains, 6–19 x 1.5–3 μ ; the other shorter, septate, not tapered or slightly enlarged at the tip, up to 35 x 6 μ , with endoconidia hyaline to pale brown, barrel-shaped, smooth, in long chains, 6–9 x 4–6 μ (Plate 2, Fig. 5).

Illustrations: Hedcock, G. G. 1906. *Mo. Bot. Gard. Ann. Rpt.* **17**: 59–114, Plate 3, Fig. 5, and Plate 5, Figs. 3–5; Davidson, R. W. 1935. *Jour. Agr. Res.* **50**: 789–807, Fig. 2, A–C; Moreau, M., and Mme. F. 1952. *Revue de Mycologie* **17**: 141–153, Figs. 1–4; Luc, M. 1952. *Revue de Mycologie*, Suppl. Colonial **17**: 10–16, Figs. 1 and 2.

This species was first described from hardwood lumber in Texas and Arkansas (Hedcock, 1906; TYPE, BPI; isotypes, BPI, FH, NY). It is one of the most common species found on hardwood logs and lumber in the southern states (Davidson, 1935; Verrall, 1939). The type of *Endoconidiophora bunae* Kitajima (1936) from Japan has been destroyed but cultures obtained from near the type locality (JH-74 and 75 = B-33 and 34 of K. Aoshima) proved to be *C. moniliformis*. *Ceratocystis wilsoni* Bakshi (1951a) described from oak in Scotland is also a synonym (IMI-20163, lectotype; culture JH-67 = S-97 from FPRL-E) as was indicated by Moreau, C. (1952). Luc (1952) separated the species into four forms based on differences in perithecial morphology, endoconidia, and endoconidiophores. These are *f. typica*, *f. davidsonii*, *f. pycnanthi* (on wood of *Pycnanthus kombo* Warb., Myristicaceae from Cameroons, Africa), and *f. theobromae* (on the roots

of cacao from Madagascar). This latter form has been studied cytologically by F. Moreau, (1952).

C. moniliformis is similar to *C. fimbriata* and *C. variospora*. It differs in having bases which are often pear-shaped and ornamented with short spines. Both *C. fimbriata* and *C. variospora* produce dark brown subglobose conidia, a spore form not found in *C. moniliformis*.

II. *Ascospores elongate-ellipsoid, cylindric, sometimes curved*

B. *Ascospores up to 12 μ long*

6. CERATOCYSTIS PARADOXA (Dade) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratosomella paradoxa Dade, Brit. Mycol. Soc. Trans. 13: 191. 1928.

Ophiostoma paradoxum (Dade) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408. 1934.

Endoconidiophora paradoxa (De Seynes) Davids., Jour. Agr. Res. 50: 802. 1935.

Sporoschisma paradoxum De Seynes, Recherches pour servir á d'histoire naturelle des vegetaux inferieurs 3(1): 30. 1886.

Perithecia partially to completely immersed in the substratum, the bases pale brown, globose, 190–240 μ in diameter or “200–350 μ ” (Dade), ornamented with numerous, irregularly shaped, knobbed appendages, dark brown to black at the tip, pale brown at the base, up to 50 μ long, 5 μ in diameter at the base and up to 20 μ at the tip; necks black, pale brown at the apex, stout, 700–1400 μ long, 45–60 μ in diameter at the base and 24–35 μ at the tip; ostiolar hyphae hyaline to pale brown, erect, numerous, tapered to a point, septate, up to 150 x 3 μ , occasionally a few hyphae occurring below the apex; asci not seen; ascospores “hyaline, ellipsoid, or frequently with unequally curved sides, in a fatty matrix, 7–10 x 2.5–4 μ ” (Dade).

Cultures hyaline to grayish at first with abundant floccose aerial mycelium, quickly becoming greenish black in the center with a grayish margin up to 15 mm. wide; whole colony becoming dark greenish black in 10 days; undersurface of the agar dark greenish black; growth rapid, more than 45 mm. in 5 days; odor not distinctive or of banana oil. Hyphae hyaline to pale brown, branched, thin-walled, septate, 2–8 μ in diameter. Endoconidiophores hyaline to pale brown, elongated, slender, urn-shaped, thin-walled, septate, up to 250 μ long, 4–8 μ in diameter at the base and 2–4 μ at the tip; endoconidia cylindrical to ellipsoid, hyaline to brown, in chains, 6–24 x 2–5.5 μ (Plate 2, Fig. 1). Conidiophores hyaline, thin-walled, relatively short hyphal side branches, up to 30 x 2–3 μ , with conidia brown, ovoid to oval, thick-walled, smooth, in chains, 11–24 x 8–19 μ .

Illustrations: Dade, H. A. 1928. Brit. Mycol. Soc. Trans. 13: 184–194, Plates X–XII.

C. paradoxa is the perfect stage of *Thielaviopsis paradoxa* (De Seynes) von Höhn. Perithecia were first described from cacao husks from the Gold Coast (Dade, 1928; IMI-41301, lectotype; culture JH-34 from CBS). The species is widespread in the tropics and subtropics on a variety of substrates. For its distribution see Imperial Mycological Institute, Distribution Maps of Plant Diseases, No. 142. 1947.

C. paradoxa and the next species, *C. radicola*, are differentiated

from the other species of *Ceratocystis* with elongate ascospores because of the peculiar hornlike appendages on the bases of the perithecia. The perithecia of the two species are very similar, varying only in the ostiolar hyphae. In *C. paradoxa* they are long and sharply pointed; in *C. radiculicola* they are shorter and blunt. The imperfect stages differ in that *C. paradoxa* produces large brown ovoid to subglobose conidia in chains; in *C. radiculicola*, on the other hand, they are produced singly at the tips of sympodially branched conidiophores.

7. *CERATOCYSTIS RADICICOLA* (Bliss) C. Moreau, *Revue de Mycologie*, Suppl. Colonial **17**: 22. 1952.

Ceratostomella radiculicola Bliss, *Mycologia* **33**: 468. 1941.

Ophiostoma radiculicola (Bliss) von Arx, *Antonie van Leeuwenhoek* **18**: 211. 1952.

Perithecia partially or completely immersed, the bases pale brown, globose, up to 300 μ in diameter, ornamented with few to many appendages with irregularly shaped tips, up to 85 x 8 μ ; necks slender, black, hyaline at the tip, up to 800 μ long, 40–60 μ in diameter at the base and 25–35 μ at the tip; ostiolar hyphae numerous, hyaline, slightly tapered but blunt at the tip, up to 60 x 3 μ ; asci not seen; ascospores elongate-ellipsoid, often flattened on one side, slightly tapered at one end, 7–12 x 2.5–3.5 μ (Plate 1, Fig. 13).

Cultures hyaline at first with abundant aerial mycelium, rapidly becoming greenish gray; numerous conidia produced after a few hours; undersurface of the agar light olive-green; growth rapid, more than 45 mm. in 5 days; odor of banana oil. Aerial hyphae hyaline to pale brown, branched, thin-walled, septate, often growing together in clusters of 2–4 hyphae, 2–6 μ in diameter; submerged hyphae similar except more interwoven and contorted. Endoconidiophores hyaline to pale brown, thin-walled, septate, unbranched, tapered, 125–250 μ high, 6–8 μ in diameter at the base and 3–5 μ at the tip, with endoconidia hyaline to pale brown, cylindrical, truncate to rounded at the ends, thin-walled, 6–22 x 4–5 μ , often formed in chains. Conidiophores hyaline, septate, sympodially branched, up to 75 μ tall, with individual branches 5–15 x 2–3 μ , the conidia borne singly, dark brown to black, globose to ovoid, thick-walled, smooth to rough-walled, 11–29 x 9–16 μ .

Illustrations: Bliss, D. E. 1941. *Mycologia* **33**: 468–482, Figs. 1–7, 10.

This species was isolated from date palms in California (Bliss, 1941; TYPE, BPI; cultures JH-3 from CBS, and JH-72 from BPI, both from type culture). It is associated with the wilting and the death of mature date palms.

C. radiculicola and the preceding species, *C. paradoxa*, have peculiar hornlike appendages (Plate 4, Fig. 1) on the bases of the perithecia and are thus easily distinguished from other species of *Ceratocystis*. The perithecia of both species are very similar but differ in that *C. radiculicola* has ostiolar hyphae up to 60 μ long which are blunt at the tips. In *C. paradoxa* the ostiolar hyphae are mostly longer and pointed. The large, dark-colored conidia of *C. radiculicola* are produced singly on sympodially branched conidiophores, in *C. paradoxa* they are produced in chains on unbranched conidiophores.

8. *Ceratocystis fagacearum* (Bretz) Hunt, comb. nov.*Endoconidiophora fagacearum* Bretz, Phytopath. 42: 437. 1952.

Perithecia immersed in the mycelial mat with only the necks emergent, the bases black, globose, up to $360\ \mu$ in diameter, or "240–380 μ " (Bretz), covered with undifferentiated hyphae; necks black, short, stout, pale brown to hyaline at the apex, up to $400\ \mu$ long or "250–450 μ " (Bretz), 80–120 μ in diameter at the base and 60–80 μ at the tip; ostiolar hyphae pale brown to hyaline, erect, numerous, slightly tapered, septate, variable in length, up to 125 μ long and 2–3 μ in diameter at the base; asci not seen; ascospores elongate-ellipsoid, sometimes flattened on one side, 5–11 x 2.5–3.5 μ .

Cultural characters (from Henry, 1944) "mycelial mat fluffy, 1–3 mm. high, white, becoming gray to olive-green with occasional patches of tan, . . . [undersurface of the agar] darkest toward the center with irregular areas and/or radiating lines of olive-green mycelium toward the hyaline margin; [growth slow,] the diameter of 1-week-old cultures growing directly from chips of diseased wood varied from 1–2.5 cm. First transfers from such cultures were 1.8–3.2 cm. in diameter at a like age; [optimum temperature for growth is] 24–28° C.; [odor] suggesting old apple cider . . . Hyphae septate, branched, subhyaline to brown, 2.5–6 μ in diameter; sclerotia, sometimes present, tan to black, loosely knit, irregular in shape, up to 2.5 cm. in diameter; conidiophores not sharply differentiated from sterile hyphae; spore-bearing cells terminating long or short branches, subhyaline to brown, often tapering slightly toward the apex, 2.5–4.5 x 20–40 μ ; conidia 2–4.5 x 4–22 μ , endogenous, hyaline, cylindrical, truncate at each end, continuous, catenulate."

Illustrations: Bretz, T. W. 1952. Phytopath. 42: 435–437, Fig. 1; Stessel, G. J., and B. M. Zuckerman. 1953. Phytopath. 43: 65–70, Fig. 1 A–J.

C. fagacearum is the perfect stage of *Chalara quercina* Henry. Perithecia were first described from culture (Bretz, 1952; TYPE, BPI-FP 97476) and have also been found in nature (Stessel and Zuckerman, 1953; 149 from Bretz, SYRF). Cultures of this fungus have not been studied since it seemed unwise to import the living fungus into New York State where it is as yet unreported. *C. fagacearum* is the causal organism associated with oak wilt which is known from the eastern and midwestern United States (Fowler, 1953).

C. fagacearum has ascospores similar to *C. radicola* and *C. paradoxa*, but differs in perithecial morphology. The base is unornamented and the neck is short and thick. In culture *C. fagacearum* is slow growing; *C. paradoxa* and *C. radicola* are fast growing.

BB. Spores not exceeding 8 μ in length

9. *CERATOCYSTIS COERULESCENS* (Münch) Bakshi, Brit. Mycol. Soc. Trans. 33: 114. 1950.

Endoconidiophora coerulescens Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 564. 1907.

Ophiostoma coerulescens (Münch) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408. 1934. *Endoconidiophora virescens* Davids., Mycologia 36: 301. 1944.

Perithecia superficial to partially immersed, the bases black, globose to flattened on one side and appearing oval, up to $200\ \mu$ in diameter, ornamented with numerous long, slender, dark brown, thin-walled, septate hyphae up to $250 \times 5\ \mu$ (Plate 4, Fig. 4); necks black, hyaline at the apex, up to $750\ \mu$ long, $35\text{--}50\ \mu$ in diameter at the base and $10\text{--}20\ \mu$ at the tip; ostiolar hyphae hyaline, spreading, tapered, $10\text{--}20$ in number, $20\text{--}30 \times 1.5\text{--}2.5\ \mu$; asci not seen; ascospores elongate-ellipsoid, rounded at the ends, often flattened on one side, rarely curved, $6\text{--}8 \times 2\text{--}3\ \mu$.

Cultures hyaline at first, becoming greenish black, with sparse to abundant aerial mycelium; endoconidia produced after a few hours; perithecia appearing in 3–5 days, maturing in 14 days; undersurface of the agar dark greenish black; growth rapid, 45 mm.; odor of banana oil or not distinctive. Hyphae light to dark brown, branched, thin-walled, septate, $2\text{--}6\ \mu$ in diameter. Endoconidiophores of two kinds, one erect, brown, unbranched, septate, thin-walled, tapered, up to $200\ \mu$ tall, $6\text{--}10\ \mu$ in diameter at the base and $3\text{--}5\ \mu$ at the tip, with endoconidia hyaline, cylindrical, with truncate or rounded ends, thin-walled, smooth, often in chains, $5\text{--}30 \times 2.5\text{--}3.5\ \mu$; the other erect, brown, unbranched, thin-walled, septate, not tapered, up to $150 \times 5\text{--}6\ \mu$ with endoconidia hyaline, oval to barrel-shaped, thin-walled, smooth, often in chains, $5\text{--}8 \times 4.5\text{--}5.5\ \mu$.

Illustrations: Münch, E. 1907. Naturw. Ztschr. f. Forst. u. Landw. 5: 531–573, Figs. 20–25; Lagerberg, T., G. Lundberg, and E. Melin. 1927. Sv. Skogsvårdsf. Tidskr. 25: 145–272, 561–691, Figs. 22–26; Siemaszko, W. 1939. Planta Polonica 7(3): 1–52, Fig. 1D, Plate I, Figs. 9–13; Bakshi, B. K. 1951. Commonw. Mycol. Inst., Mycol. Paper 35: 1–16, Fig. 1, and Plate I; Davidson, R. W. 1953. Mycologia 45: 579–586, Fig. 3.

This species was first described from stained spruce wood in Germany. No type or authentic specimens have been located. In Europe it has also been reported from Sweden (Lagerberg *et al.*, 1927), Poland (Siemaszko, 1939), Russia (R.A.M. 14: 68), Spain (R.A.M. 24: 392), Italy (R.A.M. 31: 464), and Scotland (Bakshi, 1951a; culture JH-49 = S-97 of FPRL-E). In the United States Davidson (1935) and Verrall (1939) report the species as common on hardwood lumber in the South. Davidson (1944) elevated the form on hardwood to specific rank. Hepting (1944) reports this form as the cause of sapstreak, a killing disease of sugar maple. A study of dried material and cultures of *Endoconidiophora virescens* Davids. (TYPE, BPI-FP 94161; culture JH-51 = 22523, FPRL-C; cultures JH-80 through 86 = M-33 through 37, 39, and 40 from Southeastern Forest Experiment Station, Asheville, N. C.) indicates no morphological difference between this species and *C. coerulescens* and it is considered a synonym of the latter species. In a letter with regard to this, Davidson writes: "*E. coerulescens* and *E. virescens* are going to be difficult to keep as separate species on a morphological basis. Since publishing on these species I have studied several forms of *E. coerulescens* from conifer lumber here in the West and have tentatively concluded that it is an exceedingly variable species—that is, in cultural characteristics, presence or absence

of perithecial development, and size and shape of conidiophores and conidia. However, I still believe the form on hardwoods in the South is distinct and I wish . . . to keep it separate, at least on a variety or form basis. This I believe would tend to avoid too much confusion in the future. I feel that typical *E. coerulescens* was not encountered by me while I was working in the South. In this part of the country [Colorado] I have encountered only *E. coerulescens*." The form on Douglas fir in the western United States has been designated as *E. coerulescens* Münch f. *douglasii* Davids. (1953) (culture JH-54a = 70703 from Davidson; 144 and 150 SYRF).

This species has ascospores similar in shape to the preceding three species, *C. radiculicola*, *C. paradoxa* and *C. fagacearum* but shorter and more slender. The ostiolar hyphae differ in that they are spreading, rather than erect, and are shorter than in the above three species. The dense covering of long, dark colored, slender, unbranched hyphae ornamenting the base of the perithecium is distinctive.

10. CERATOCYSTIS AUTOGRAPHA Bakshi, Ann. Bot., n.s. 15: 55. 1951.

Perithecia superficial, the bases black, globose, 60–120 μ in diameter, or "ventral diameter and height vary from 88–182 μ and 78–165 μ respectively" (Bakshi), ornamented with a few black, unbranched, moderately thick-walled, septate hyphal hairs 20–100 \times 2–3 μ ; necks black, sometimes bent, up to 650 μ long, 18–32 μ in diameter at the base and 8–12 μ at the tip; ostiolar hyphae hyaline, untapered, thin-walled, somewhat spreading, 8–20 in number, up to 55 \times 1.5 μ ; asci not seen; ascospores cylindrical, rounded at the ends, not curved, 3–4 \times 1–1.5 μ (Plate 1, Fig. 14).

Cultures hyaline to grayish with a few black radial streaks; mostly appressed with sparse aerial growth; growth slow, about 5 mm.; odor not distinctive. Hyphae hyaline to pale brown, branched, thin-walled, septate, up to 3.5 μ in diameter, often agglutinated together into thick strands of varying diameter. Endoconidiophores brown, elongated, urn-shaped, thin-walled, 15–25 μ long, 3 μ in diameter at the base and 1.5 μ at the tip, with endoconidia hyaline, cylindrical, truncate at the ends, catenate, 4–5.5 \times 1.5 μ (Plate 2, Fig. 4). Conidiophores hyaline, septate, 50 \times 1–1.5 μ , with conidia hyaline, globose to subglobose, 1.5–2.5 \times 1.5 μ , in clusters at the tips.

Illustrations: Bakshi, B. K. 1951. Ann. Bot., n.s. 15: 53–61, Figs. 1, 2 and 4, and Plate VI, Figs. 4–7.

This fungus was isolated in Scotland from the galleries of bark beetles (*Dryocoetes autographus* and *Hylurogops palliatus*) on Japanese larch (IMI-20162, lectotype; culture JH-63 = S-99 from FPRL-E).

C. autographa is the only species in the endoconidial group with short cylindric ascospores.

Section 2. Species with *Leptographium* or *Graphium* imperfect stages.

I. Conidial stage a *Leptographium* (*Grosmannia* group)

A. Ostiolar hyphae absent

11. CERATOCYSTIS PENICILLATA (Groszm.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella penicillata Groszm., Hedwigia 72: 190. 1932.

Grosmannia penicillata (Groszm.) Goid., R. Staz. Pat. Veg. Bol., Rome, n.s., 15: 156. 1935.

Ophiostoma penicillatum (Groszm.) Siem., Planta Polonica 7(3): 24. 1939.

Perithecia superficial on the surface of the bark or wood, the bases black, globose, up to $200\ \mu$ in diameter or " $250\text{--}300\ \mu$ " (Grosmann), ornamentation lacking; necks black, relatively stout, up to $500\ \mu$ long, $35\text{--}50\ \mu$ in diameter at the base and $17\text{--}30\ \mu$ at the tip, or " $300\text{--}500\ \mu$ lang und etwa $50\ \mu$ dick" (Grosmann); ostiolar hyphae absent; asci not seen; ascospores with a thick gelatinous sheath, ellipsoid, often weakly curved, sometimes hat-shaped due to sheath forming a brim, $5\text{--}6 \times 3\text{--}4\ \mu$ including sheath (Plate 1, Fig. 4), or " $6.5 \times 2.3\ \mu$ " (Grosmann).

Cultures hyaline at first, turning brownish green after a few days, with a loose cottony appearance; growth rapid, 45 mm.; odor not distinctive. Aerial hyphae pale brown, branched, thin-walled, septate, $2\text{--}5\ \mu$ in diameter; submerged hyphae (in wood) dark brown, $2\text{--}8\ \mu$ in diameter. Conidiophores of the *Leptographium* type, the stalks brown, moderately thick-walled, 4–7 septate, $150\text{--}500 \times 4\text{--}8\ \mu$, the heads hyaline to pale yellow, up to $200\ \mu$ in diameter, with conidia hyaline, cylindrical to ellipsoid, $3\text{--}5.5 \times 1.5\text{--}2\ \mu$, in freshly isolated cultures conidia "hyalin, anfangs oval, später sichelförmig gekrümmt, und haben ein Länge von $11\text{--}12\ \mu$ bei einer mittleren Breit von $3\text{--}3.5\ \mu$ " (Grosmann). Conidiophores of the *Cephalosporium* type hyaline to pale brown, simple to branched, thin-walled, septate, $10\text{--}20 \times 2\text{--}3\ \mu$, with conidia hyaline, ellipsoid to oval, $3\text{--}6 \times 2\text{--}4\ \mu$, or " $6\text{--}14 \times 4\text{--}6\ \mu$ " (Grosmann).

Illustrations: Grosmann, H. 1932. Hedwigia 72: 183–194, Figs. 1, 2, 5, 6; Siemaszko, W. 1939. Planta Polonica 7(3): 1–52, Figs. 1A, Plate II, Figs. 1–4; Mathiesen, Aino 1950. Oikos 2: 275–308, Figs. 1, 2.

This species was first reported from Germany associated with bark beetles on *Picea excelsa* (Grosmann, 1932; culture JH-9 from CBS). It has also been reported from Poland (Siemaszko, 1939) and Sweden (Mathiesen, 1950; 7–1, 7–2, 9–2, AM-K). Several strains of this fungus were encountered in Sweden by Aino Mathiesen (1950) and of these she recognizes four forms based on differences in the imperfect stages, cultural characters, and associated insects. The forms described are f. *typica*, f. *chalcographi*, f. *palliat*, and f. *pini*. The latter two forms have not developed perithecial stages.

Although freshly isolated cultures of this fungus are distinctive, isolates retained in culture for a long period of time gradually change both in appearance and in production of perithecia and imperfect stages. Mathiesen (1950) reports that the *Leptographium* stage deteriorates in culture. The cultural characteristics and the measurements of the conidia and conidiophores given in the above description were taken from a culture received from the Centraalbureau voor Schimmelcultures. Since this culture has been retained for several years, these details may not be characteristic.

C. penicillata, and the following two species, *C. piceaperda* and *C. serpens*, have perithecia, ascospores, and imperfect stages which are very similar. *C. penicillata* and *C. piceaperda* differ from *C. serpens* in the longer ascospores. *C. penicillata* may be differentiated from *C. piceaperda* by the wider ascospores and the shorter perithecial necks. No. 46, *Ceratostomella imperfecta*, known only from the original description, has perithecia similar to *C. penicillata*. The *Haplographium* imperfect stage attributed to *C. imperfecta* is probably the same as the *Leptographium* of *C. penicillata* and this species may be a synonym of *C. penicillata*. No. 41, *Ceratostomella buxi*, an imperfectly known species, is also similar.

12. CERATOCYSTIS PICEAPERDA (Rumb.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella piceaperda Rumb., Jour. Agr. Res. 52: 432. 1936.

Grosmannia piceaperda (Rumb.) Goid., R. Staz. Pat. Veg. Bol., Rome, n.s., 16: 255. 1936 (footnote).

Ophiostoma piceaperdum (Rumb.) von Arx, Antonie van Leeuwenhoek 18: 211. 1952.

Perithecia superficial, the bases black, globose, up to 200 μ in diameter, or "90 μ –250 μ altis, 80 μ –340 μ latis" (Rumbold), unornamented; necks black, relatively stout, up to 900 μ long, 20–40 μ in diameter at the base and 15–25 μ at the tip; ostiolar hyphae absent; asci not seen; ascospores with a thick gelatinous sheath, ellipsoid, often slightly curved, sometimes hat-shaped, 4–6 x 2.5–3 μ including sheath, or "ellipsoideis, 3.6 μ –4.7 μ longis, 1.0 μ –2.4 μ latis" (Rumbold).

Cultures hyaline to white at first, with abundant aerial mycelium, becoming brown to black in some plates, remaining hyaline to gray in others; mycelial conidia formed from the first; conidiophores of the *Leptographium* type appearing in 2–3 days; perithecia not found in culture, "the fungus developed mature perithecia in 14 days when growing on steam-sterilized pine" (Rumbold); growth rapid, 40 mm.; odor not distinctive. Hyphae brown, septate, thin-walled, branched, up to 8 μ in diameter. Conidiophores of the *Leptographium* type, the stalks brown, thick-walled, 5–9 septate, up to 300 x 8 μ , terminating in a cluster of hyaline branches, the head up to 80 μ in diameter, with conidia hyaline, cylindrical to ellipsoid, 4.5–9.5 x 2–3 μ . Simple hyaline conidiophores up to 20 x 3 μ with conidia like those above.

Illustrations: Rumbold, C. T. 1936. Jour. Agr. Res. 52: 419–437, Figs. 8–10.

C. piceaperda was found in the provinces of Nova Scotia and Quebec associated with bark beetle attacks on spruce (lectotype, BPI-240CTR; NY; culture JH-13 from CBS). The wood of spruce is stained a pale gray in the vicinity of the bark beetle attacks.

C. piceaperda is very similar to *C. penicillata* and varies only in having narrower ascospores and slightly longer perithecial necks. These species are so similar in all respects that further study may show that *C. piceaperda* is a synonym of *C. penicillata*.

13. CERATOCYSTIS SERPENS (Goid.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Grosmannia serpens Goid., R. Staz. Pat. Veg. Bol., Rome, n.s., 16: 42. 1936.

Ophiostoma serpens (Goid.) von Arx, Antonie van Leeuwenhoek 18: 211. 1952.

Perithecia not seen, "peritheciis superficialibus vel subimmersis atris globosis, 300–420 μ diam. circiter, pilis raris, nigris in parte inferiore perithecii praeditis; rostro rigido, nigro, 400–700 μ longo, inferne 33–65 μ , superne 21–39 μ crasso, apice sine ciliis hyalinis; ascis ovalibus, irregularibus, cito diffluentibus; ascosporis hyalinis, ellipsoidibus, apicibus rotundatis vel leniter truncatis, 3.3–4.8 x 1.8–2 μ " (Goidanich).

Cultures hyaline at first becoming black; mycelium appressed except for numerous erect conidiophores of the *Leptographium* type; rate of growth rapid, 45 mm.; odor not distinctive. Hyphae sinuous, hyaline to brown, branched, thin-walled, frequently septate, 2–15 (–25) μ in diameter; hyphae from which conidiophores arise dark brown, thick-walled, septate, 10–14 μ in diameter. Conidiophores as in *Leptographium* (Plate 3, Fig. 4), the stalks dark brown, thick-walled, 3–7 septate, 65–200 x 8–12 μ or "320–750 μ longis 12–18 μ latis" (Goidanich), ramified heads globose, up to 80 μ in diameter and made up of repeatedly branched hyphae, pale brown at the base, hyaline at the tips, with conidia hyaline, ellipsoid to ovate, often pointed at one end, 4–8.5 x 2.5–3 μ or "3.2–5 x 2.5–2.7 μ " (Goidanich).

Illustrations: Goidanich, G. 1936. R. Staz. Pat. Veg. Bol., Rome, n.s., 16: 26–60, Figs. 1, 2, 4, 7–16, and Plate I, Figs. 1–7; Siemaszko, W. 1939. Planta Polonica 7(3): 1–52, Plate 5, Fig. 3.

C. serpens was described from *Pinus sylvestris* in Italy (culture JH-42 from CBS). The imperfect stage has also been recorded from Poland (Siemaszko, 1939). A very similar imperfect stage has been reported on Scots pine in Sweden (Mathiesen, 1950) and provisionally named *O. penicillata*. No herbarium material was obtained of *C. serpens* nor is it known if a type collection has been preserved.

This species is very similar to No. 11, *C. penicillata*, and may actually be a synonym of the latter species. Based on Goidanich's description the species differs from *C. penicillata* only in its smaller ascospores. No. 41, *Ceratostomella buxi*, an imperfectly known species, also appears to be similar.

14. *Ceratocystis rostracylindrica* (Davids.) Hunt, comb. nov.

Ceratostomella (*Grosmannia*) *rostracylindrica* Davids., Mycologia 34: 658. 1942.

Ophiostoma rostracylindrica (Davids.) von Arx, Antonie van Leeuwenhoek 18: 212. 1952.

Perithecia superficial to partially immersed, the bases black, globose, up to 240 μ in diameter or "about 300 μ " (Davidson), unornamented; necks black, rounded at the tip, up to 600 μ long, 40–60 μ in diameter at the base and 25–40 μ at the tip; ostiolar hyphae absent; asci not seen; ascospores with a thick, almost transparent sheath, oval to ellipsoid in outline including the sheath, or ellipsoid to curved, some almost crescent-shaped without the sheath, 2.5–4 x 1–1.5 μ including the sheath (Plate 1, Fig. 5).

Cultures growing very slowly, white at first, becoming dark brown to black or "dark brownish gray" (Davidson), with little or no aerial mycelium; "perithecia develop slowly, maturing in 4 to 6 weeks" (Davidson); undersurface of the agar black in the center; growth slow, 5 mm.; odor not distinctive. Hyphae hyaline at first, becoming brown, branched, thin-walled, septate, variable in size, 1–3 (–5) μ in

diameter. Conidiophores of the *Leptographium* type, the stalks brown, thick-walled, 4-7 septate, 100-300 x 2.5-3.5 (-8) μ , ramified heads hyaline, up to 50 μ in diameter, with conidia hyaline, elliptical to ovate, 3-6 x 1-2 μ . Mycelial conidia produced directly on the vegetative hyphae, of various sizes and shapes, 3-16 x 1.5-6 μ .

Illustrations: Davidson, R. W. 1942. *Mycologia* 34: 650-662, Figs. 2B and 3A-E.

C. rostracylindrica is known only from the type culture or subcultures of it (culture JH-77 from BPI). No type was designated for this species and the only perithecia preserved are on some slides prepared by Davidson (lectotype, BPI, slides no. 1881-1-148). The species was isolated from oak in Connecticut and is not of importance either as a staining organism or as a pathogen on oak.

This species, *C. rostracylindrica*, differs from the preceding three species in the smaller ascospores and by its slow growth in culture.

AA. Ostiolar hyphae present

15. CERATOCYSTIS POLONICUM (Siem.) C. Moreau, *Revue de Mycologie*, Suppl. Colonial 17: 22. 1952.

Ophiostoma polonicum Siem., *Planta Polonica* 7(3): 32. 1939.

Perithecia superficial, the bases black, globose to somewhat flattened, up to 300 μ in diameter or "250-450 μ " (Siemaszko), often covered with undifferentiated hyphae; necks slender, black, hyaline at the apex, up to 1800 μ long or "340-1200 μ " (Siemaszko), 40-55 μ in diameter at the base and 15-25 μ at the tip; ostiolar hyphae not seen, "mit 11-16 langen, hyalinen, allmählich zugespitzten Zilien, 20-60 x 2 μ " (Mathiesen, 1951) or "apice 12-20 filamentis hyalinis, 30-70 [μ] longis, acutis coronato" (Siemaszko; asci not seen; ascospores cylindrical to ellipsoid, often flattened on one side, sometimes hat-shaped, with a thick, almost transparent sheath around each spore, 4.5-7 x 2-2.5 μ , including slime sheath).

Cultures pale greenish gray, becoming dark greenish gray in older cultures, with a floccose, loosely interwoven aerial mycelial mat; scattered perithecia forming after 4-5 days, maturing in 2 weeks; growth rapid, 45 mm.; odor not distinctive. Hyphae hyaline at first, becoming brown, branched, thin-walled, septate, loosely interwoven, 2.5-8 μ in diameter. Conidia when present on conidiophores of the *Leptographium* type, the stalks erect, brown, thick-walled, 4-6 septate, 100-300 μ tall or up to "500 μ longis" (Siemaszko) and 5-8 μ in diameter, the head white to pale yellowish, up to 75 μ in diameter, with conidia hyaline, ellipsoid to broadly oval, 3-6 x 2-3.5 μ or "4.4-8 x 2-4(5) μ " (Siemaszko).

Illustrations: Siemaszko, W. 1939. *Planta Polonica* 7(3): 1-52, Fig. 1E, Plate IV, Figs. 1-10.

This species was first described from Poland on the bark of living spruce trees in association with the bark beetle *Ips typographus* (Siemaszko, 1939; culture JH-43 from CBS). It has also been reported from Sweden (Mathiesen, 1951). *C. polonicum* is not important economically either in Sweden or Poland, although it does cause a discoloration of wood.

The presence of ostiolar hyphae is one character by which *C. polonicum* differs from the other species with a *Leptographium* stage. The next species, *C. leptographioides*, also has ostiolar hyphae but it can be clearly distinguished from *C. polonicum* by the small perithecia with short necks and the quadrangular ascospores. Mathiesen (1951) points out that this species represents a transitional form between those species with a *Leptographium* for the imperfect stage (the *Grosmannia* group) and those species with imperfect stages other than *Leptographium* (the *Ophiostoma* group). The perithecial characters of *C. polonicum* are more like those found among the species in the *Ophiostoma* group, but the species belongs in the *Grosmannia* group, based on cultural characters and the imperfect stage.

16. ***Ceratocystis leptographioides*** (Davids.) Hunt, comb. nov.

Ceratostomella (*Grosmannia*) *leptographioides* Davids., Mycologia 34: 657. 1942.
Ophiostoma leptographioides (Davids.) von Arx, Antonie van Leeuwenhoek 18: 211. 1952.

Perithecia superficial to immersed, the bases dark brown to black, globose, up to 150 μ in diameter, unornamented except for undifferentiated hyphae; necks black, hyaline at the apex, short, up to 150 μ long or "150–180 μ long" (Davidson), 25–35 μ in diameter at the base and 15–25 μ at the tip; ostiolar hyphae hyaline, numerous, not spreading, pointed at the tips, septate, thin-walled, up to 60 \times 2–3 μ ; asci not seen; ascospores with gelatinous sheath forming flanges or brims, rectangular, sometimes with non-parallel sides, a few hat-shaped, 5.5–7 \times 2–3 μ , or "6–7.5 \times 2.8–3.8 μ " (Davidson).

Cultures white to gray at first, gradually becoming brown; aerial mycelium sparse and cobwebby at first, soon appressed and the surface covered with conidiophores of the *Leptographium* type; perithecia appearing in 1 week, maturing in 3–4 weeks; growth slow to intermediate, 7–12 mm.; odor not distinctive. Hyphae hyaline to pale brown, branched, thin-walled, septate, interwoven, 1.5–6 μ in diameter. Conidiophores of the *Leptographium* type, the stalks erect, brown, arising from enlarged dark-colored hyphal cells, moderately thick-walled, 2–5 septate, up to 200 \times 8 μ , heads up to 50 μ in diameter, with conidia hyaline, cylindrical to ellipsoid, 4–7.5 \times 2–3 μ .

Illustrations: Davidson, R. W. 1942. Mycologia 34: 650–662, Fig. 1D–G, and 2A.

C. leptographioides is known from cultures isolated from oak in Virginia and Ohio (TYPE, BPI-FP 59117; cultures JH-70 = 71596 BPI, and JH-78 = M-101, BPI). It is not known to be important either as a staining fungus or as a pathogen.

This species differs from other species with *Leptographium* as the imperfect stage because of the small perithecia with short necks, the erect, pointed ostiolar hyphae, and the unsymmetrically quadrangular ascospores. The next two species, *C. olivaceum* and *C. obscura*, species with a Graphium-like imperfect stage, also have these perithecial characters and, together with *C. leptographioides*, form a distinct group in the genus *Ceratocystis*. Aside from the imperfect stage, *C. leptographioides* may be separated from *C. olivaceum* by the shorter perithecial necks, and from *C. obscura* by the larger ascospores. Com-

pare also No. 44, *Ceratostomella comata*, an imperfectly known species.

This group of species is an additional example of the lack of agreement between the perithecial morphology and the imperfect stages. Based on the imperfect stage, *C. leptographioides* belongs in the *Grosmannia* group; on perithecial morphology it belongs in the *Ophiostoma* group.

II. Conidial stage a *Graphium* (*Ophiostoma* group in part)

B. Ascospores hat-shaped or quadrangular, with gelatinous appendages, either in the form of brims or flanges

C. Perithecia with short necks, usually not over 400 μ long

17. *Ceratocystis olivacea* (Mathiesen) Hunt, comb. nov.

Ophiostoma olivaceum Mathiesen, Sv. Bot. Tidskr. 45: 212. 1951.

Perithecia superficial, the bases dark brown to black, up to 135 μ in diameter or "182 μ hoch, 177 μ breit" (Mathiesen), unornamented except for a few undifferentiated hyphae; necks relatively short and stout, up to 400 μ long, 25–35 μ in diameter at the base and 20–30 μ at the tip; ostiolar hyphae pale brown, numerous, tapered to a blunt point, thin-walled, septate, irregular in length, 40–80 \times 2.5–3.5 μ ; asci not seen; ascospores quadrangular due to gelatinous sheath which forms two unsymmetrically placed brims, a few hat-shaped, 5–6.5 \times 3–4 μ including the sheath.

Cultures pale at first becoming dark olive-green to black, with sparse to no aerial mycelium; undersurface of the agar becoming dark black; growth intermediate, 15 mm.; odor not distinctive. Aerial hyphae hyaline, sparse, branched, thin-walled, septate, 1–3 μ in diameter; submerged hyphae hyaline to pale brown, branched, interwoven, of various sizes, 1–8 μ in diameter. Conidiophores of the *Graphium* type, the stalks stout, thick, the lower part brown to black, the upper part hyaline, up to 350 \times 100 μ or "316(255–368) μ altis, 74(43–142) μ crassis" (Mathiesen), the heads consisting of ramified conidiophores, up to 300 μ in diameter or "180–1200 μ diam." (Mathiesen), with conidia hyaline, broadly oval, 3–4 \times 2–3 μ . Mycelial conidia produced at the tips of the hyphae or directly on the vegetative mycelium, hyaline, broadly oval, 3.5–6.5 \times 3 μ .

Illustrations: Mathiesen, Aino. 1951. Sv. Bot. Tidskr. 45: 203–232, Fig. 2, a–g.

This species was described from Sweden associated with the bark beetle *Aconthocinus aedilis* on pine (Mathiesen, 1950, 1951; 4–3, AM-K). The species has been found in New York (148, SYRF) associated with ambrosia beetles on white pine. No type has been designated for this species but herbarium material and cultures are preserved at Statens Skogsforskningsinstitut, Experimentalfältet, Sweden. The wood is stained a weak blue around the tunnel system but the species has little importance as a staining fungus.

C. olivacea and the next species, *C. obscura*, are very similar both in perithecial morphology and in imperfect stages. They differ in that *C. olivacea* has ostiolar hyphae which are longer and ascospores which are larger than in *C. obscura*. These two species and *C. leptog-*

graphioides comprise a distinct group in the genus, based on perithecial characters and ascospores. No. 44, *Ceratostomella comata*, a species known only from the original description, also belongs in this group of species, and apparently differs from *C. olivacea* in having smaller perithecial bases and coremia.

18. ***Ceratocystis obscura*** (Davids.) Hunt, comb. nov.

Ceratostomella obscura Davids., Jour. Agr. Res. **50**: 798. 1935.

Ophiostoma obscura (Davids.) von Arx, Antonie van Leeuwenhoek **18**: 211. 1952.

Perithecia known only from culture, superficial to immersed, the bases brown to black, globose, 80–120 μ in diameter or “90 μ to 150 μ ” (Davidson), unornamented; necks black, short, up to 400 μ long, 20–30 μ in diameter at the base and 15–25 μ at the tip; ostiolar hyphae pale brown, numerous, erect, pointed, septate, of variable length, up to 40 μ long and 2–3 μ in diameter at the base; asci not seen; ascospores with gelatinous sheath forming brims, quadrangular with two unsymmetrically placed brims, or sometimes hat-shaped with one brim, 4–5.5 x 2–2.5 μ including brims (Plate 1, Fig. 2).

Cultures “hyaline at first, appressed to the substratum, becoming light gray-green in 4 to 5 days, never very dark; conidial growth at first almost invisible, graphia soon forming; perithecia slow to form, often absent, . . . growth, 17 mm. in 5 days” (Davidson). Hyphae hyaline to pale brown, branched, thin-walled, septate, 1–5 μ in diameter. Conidiophores variable, “brushlike branches of the mycelium” (Davidson) or distinct coremia of the *Graphium* type, the stalks light brown, erect, flaring to a hyaline apex, up to 300 x 36 μ or “100 μ to 700 μ high by 15 μ to 70 μ thick, often originating from [a] single hypha” (Davidson), the heads consisting of ramified conidiophores imbedded in a slime drop up to 100 μ in diameter, with conidia hyaline, cylindrical to ellipsoid, quite variable, 4.5–9 x 3–4.5 μ .

Illustrations: Davidson, R. W. 1935. Jour. Agr. Res. **50**: 789–807, Fig. 1E–J.

This species was isolated from pine logs in the South (TYPE, BPI-FP 59046; slide No. 544, Davidson, BPI; culture JH-14 from CBS). It is of little importance as a staining fungus. The type collection contains only the imperfect stages.

C. obscura differs from the preceding species, *C. olivacea*, in the shorter ostiolar hyphae and the smaller ascospores. The conidial stages of *C. obscura* are similar to those of the following species, *C. ips*, a feature noted by Davidson (1935). The imperfect stage thus represents a transitional form between *Leptographium* and *Graphium*. Compare also with No. 44, *Ceratostomella comata*, an imperfectly known species.

CC. *Perithecia with long necks, usually over 500 μ long*

19. ***CERATOCYSTIS IPS*** (Rumb.) C. Moreau, Revue de Mycologie, Suppl. Colonial **17**: 22. 1952.

Ceratostomella ips Rumb., Jour. Agr. Res. **43**: 864. 1931.

Ophiostoma ips (Rumb.) Nannf., Sv. Skogsvårdsf. Tidskr. **32**: 408. 1934.

Grosmannia ips (Rumb.) Goid., R. Staz. Pat. Veg. Bol., Rome, n.s., **16**: 51. 1936.

Perithecia superficial, the bases black, appearing brown to black under the microscope, globose, 120–200 μ in diameter or “extreme range 96 μ to 320 μ ” (Rumbold), ornamented with pale brown, thin-walled, septate hyphae attached to all sides, some bearing conidia, others continuous with the vegetative mycelium, 2.5–5 μ in diameter; necks black, sometimes bent, up to 1100 μ long, 27–40 μ in diameter at the base and 10–25 μ at the tip; ostiolar hyphae lacking; asci not seen; ascospores appearing rectangular due to flanges formed by the gelatinous sheath, 3–5 x 1.5–2.5 μ (Plate 1, Fig. 1).

Cultures producing at first a loosely interwoven, sparse, hyaline mycelium, turning brown in about 5 days, and finally black in 2 weeks; sometimes areas remaining brown due to sectoring; some isolates with mycelium completely appressed, others with a matted aerial mycelium; conidia often absent, when present, appearing in 3–5 days; perithecia forming after 1–2 weeks, maturing in about 1 week after appearance; growth rapid, 35 mm.; odor not distinctive. Aerial hyphae hyaline at first, turning pale brown to almost black with age, occurring singly and in fascicles of 2–10 hyphae, branched and interwoven, thin-walled, regularly septate, some cells swollen, 1–10 μ in diameter; submerged hyphae similar; hyphae in wood thin-walled, pale brown to black, septate, concentrated in the rays, 2–3 μ in diameter. Conidiophores sometimes absent, varying from single, septate, hyaline to pale brown hyphae with conidia borne at the tips of 2–5 ramified branches to Graphium-like structures with a definite stalk and head of ramified branches, the latter up to 300 μ high, up to 20 μ in diameter at the base and flaring to a head up to 250 μ in diameter; conidia hyaline, cylindric to ovoid, sometimes truncate, 4–8 x 1.5–3 μ .

Illustrations: Rumbold, C. T. 1931. Jour. Agr. Res. 43: 847–873, Figs. 6–8, and 1936. *Ibid.* 52: 419–437, Figs. 1–5; Nisikado, Y. and K. Yamauti. 1933. Ohara Inst. f. Landw. Forsch. Ber. 5: 501–538, Plates XLVI–LVII; Siemaszko, W. 1939. Planta Polonica 7(3): 1–52, Fig. 1F, Plate 1, Figs. 1–5.

C. ips was first described from the eastern United States associated with bark beetles on pine (lectotype BPI-202-11CTR; NY) and is widespread in the United States (Rumbold, 1936; JH-22, SYRF; JH-11 from CBS). It has also been reported from Japan (Nisikado and Yamauti, 1933), and from the following countries in Europe: Germany (3–1, AM-K), Poland (Siemaszko, 1939), and Sweden (6–1, 6–2, AM-K).

Davidson (1935) and Verrall (1939) found *C. ips* to be an important cause of log and lumber stain on pine in the South. It is associated with beetle attacks in the logs and continues to develop in lumber cut from infested logs. It may be spread to uncontaminated lumber by insects or by contact with contaminated mill equipment or lumber (Verrall, 1941).

The ascospores of *C. ips* have the “shape of quadrangular prisms, . . . not cylinders as was stated in the technical description of the eastern strains of this fungus in 1931” (Rumbold, 1936). Other species with similarly shaped ascospores are No. 34, *C. montia* and No. 20, *C. brunneo-ciliata*. *C. ips* may be differentiated from the former by the smaller perithecial bases and the presence of a Graphium-like imperfect

stage, and from the latter by the absence of ostiolar hyphae. Based on the *Leptographium*-like imperfect stage, Goidanich (1936a) placed *C. ips* in his new genus, *Grosmannia*. Siemaszko (1939) does not recognize *Grosmannia* and further points out that *C. ips* produces *Graphium*-like conidiophores. The coremia of *C. ips* represent a transitional form between *Leptographium* and *Graphium* and in the strict sense the imperfect stage cannot be placed in either of the above genera. Other species with similar imperfect stages are No. 18, *C. obscura*, No. 21, *C. galeiformis*, and No. 44, *C. comata*.

20. ***Ceratocystis brunneo-ciliata*** (Mathiesen-K.) Hunt, comb. nov.

Ophiostoma brunneo-ciliatum Mathiesen-K., Meddel. Statens Skogsforskningsinst., Sweden, 43(4): 44. 1953.

Perithecia superficial, the bases black, globose, 160–220 μ in diameter, or “192–283 μ ” (Mathiesen-Käärik), sparsely ornamented with brown, branched, septate, thin-walled hyphae scarcely differentiated from the vegetative hyphae; necks black, long and slender, up to 1400 μ long, 30–40 μ in diameter at the base and 19–24 μ at the tip; ostiolar hyphae pale brown, shaped like a corkscrew, septate, numerous, up to 70 x 3 μ ; asci not seen; ascospores rectangular in appearance, due to flanges formed by the gelatinous sheath, 4–5 x 2 μ .

Cultures, as described by Mathiesen-Käärik (1953), hyaline at first, the center becoming brownish to black after 2–3 days, or remaining hyaline for 6–7 days and then becoming black; finally all cultures becoming a brownish black or a pure black; growing cultures with a hyaline margin 1.2–1.5 mm. in width; aerial hyphae sparse and mostly appressed at first, gray-brown; secondary aerial mycelium appearing in 2 weeks, of fine, gray, sterile hyphae, mostly appressed or up to 5 mm. high; mycelial conidia sparse; coremia formed in 7–10 days in the center of the colony, new ones developing radially, some also found in irregularly arranged isolated patches; perithecia forming associated with irregular patches of coremia; growth rapid, 100 mm. in 10 days. Hyphae pale brown, branched, thin-walled, septate, 1.5–6 μ in diameter, sometimes forming agglutinated strands of 3–10 hyphae. Conidiophores of two types, the one *Cephalosporium*-like, hyaline to pale brown, septate, 10–30 x 2–3 μ , with conidia hyaline, “länglich-oval und ziemlich gross, 4.2–8.9 x 2.5–4.3 μ ” (Mathiesen-Käärik); and the other *Graphium*-like, the stalks brown, hyaline and tapered at the tip, up to 1500 x 80 μ , the heads composed of brushlike masses of hyphae imbedded in a yellowish drop of liquid extending along the upper third to half of the coremium and up to 100 μ in diameter, with conidia hyaline, cylindrical, 4.5–6 x 1–2 μ , or “länglich-zylindrisch, 4.0–6.8 x 1.2–1.9 μ ” (Mathiesen-Käärik).

Illustrations: Mathiesen-Käärik, Aino. 1953. Meddel. Statens Skogsforskningsinst., Sweden, 43(4): 1–74, Figs. 1 and 2.

C. brunneo-ciliata was described from Sweden associated with the bark beetle *Ips sexdentatus* on pine (4–1, 4–2, 5–1, 5–2, 11–2, 12–1, 13–1, 13–4, and 15–1, AM-K). No type specimens have been designated, but herbarium material and cultures are preserved at the Statens Skogsforskningsinstitut, Experimentalfältet, Sweden. This fungus

causes an intense stain of pine, but it does not always penetrate deeply into the wood.

The brown, corkscrew-shaped ostiolar hyphae of *C. brunneo-ciliata* readily separate it from the other species of *Ceratocystis* with quadrangular-shaped ascospores. No. 25, *C. clavata*, has similar ostiolar hyphae but differs in the shape of the ascospores.

21. *CERATOCYSTIS GALEIFORMIS* Bakshi, Commonw. Mycol. Inst., Mycol. Paper 35: 13. 1951.

Ophiostoma galeiformis (Bakshi) Mathiesen-K., Meddel. Statens Skogsforskingsinst., Sweden, 43(4): 47. 1953.

"Perithecia develop rarely in cultures, [the bases] globose, black, . . . ventral diameter and height vary from 182–271.6 (average 221.8) μ and 182–273 (average 218) μ respectively; [ornamented with] thick-walled, unbranched, ventral hairs, brown at base, colourless at tip, and 2–2.5 μ broad; [necks] black, 539–700 (average 638) μ long, 39.2–60.2 (average 48.6) μ broad at the base, and 15.4–28.0 (average 24.5) μ broad at the top; [ostiolar hyphae lacking; asci evanescent;] ascospores are hyaline, 1-celled, thin-walled, bean shaped, and possess a brim . . . 4–5.3 x 2.1–3 (average 4.6 x 2.5) μ with brim and 3.2–4.2 x 1.7–2.1 (average 3.6 x 1.9) μ without brim" (Bakshi).

Cultures hyaline at first, becoming dense black in the center; growth at first appressed, later with numerous fascicled aerial tufts and interwoven hyphal strands with coremia; undersurface of the agar black; growth slow, 10 mm.; odor not distinctive. Aerial hyphae hyaline, becoming brown, branched, thin-walled, septate, 1.5–5 μ in diameter, agglutinated into tapered tufts consisting of many filaments; submerged hyphae similar. Conidiophores variable, of 3 types, the first simple, hyaline, short branches of the mycelium 10–20 x 1.5–2.5 μ , with conidia hyaline, ellipsoid to oval, produced at the tips in a manner resembling *Cephalosporium*, 3–3.5 x 2–2.5 μ or "2.2–3.1 x 1.5–2 μ " (Bakshi); the second resembling *Leptographium*, the stalks brown, thick-walled, up to 8-septate, up to 300 x 3–5 μ , the heads hyaline, composed of ramified branches in a slime drop, up to 50 μ in diameter, with conidia hyaline, cylindrical to ellipsoid, 5–6 x 2.5–3 μ or "4–5.2 x 1.9–2.2 μ " (Bakshi); and the third type resembling *Graphium*, the stalks consisting of two to many agglutinated thick-walled hyphae brown to black at the base, hyaline in the upper part, up to 300 x 60 μ , the hyaline heads composed of brushlike masses of ramified hyphae imbedded in a drop of liquid up to 200 μ in diameter, with conidia as in the second type.

Illustrations: Bakshi, B. K. 1951. Commonw. Mycol. Inst., Mycol. Paper 35: 1–16, Fig. 4, Plate III, Figs. 19–22; Mathiesen-Käärik, Aino. 1953. Meddel. Statens Skogsforskningsinst., Sweden, 43(4): 1–74, Figs. 3, 5.

C. galeiformis was isolated in Scotland from the bark of *Larix kaempferi* infested with bark beetles (authentic, IMI-20168; culture JH-64 = S-100, FPRL-E). No perithecia were found on the specimens distributed by Bakshi. It has also been reported from Sweden associated with bark beetles on pine (2-2, 14-1, AM-K). Grown artificially on wood the fungus develops a grayish blue coloration which is severe

on Scots pine but not dark on spruce or larch (Bakshi, 1951a). In Sweden the fungus stains the wood a weak greenish blue around the insect tunnels.

This species has perithecia like No. 11, *C. penicillata*, and No. 12, *C. piceaperda*, but differs in having smaller ascospores and a Graphium-like imperfect stage. Cultures of *C. galeiformis* are slow growing, and the latter two species have rapidly growing cultures. It also is similar to No. 19, *C. ips*, but differs in the shape of the ascospores. Like *C. ips*, the imperfect stages of this species represent a transition between *Leptographium* and *Graphium*. Compare also No. 40, *Ceratostomella acoma*, an imperfectly known species.

BB. Ascospores bean-shaped, kidney-shaped, crescent-shaped, or like sections of an orange, lacking appendages

22. CERATOCYSTIS PICEAE (Münch) Bakshi, Brit. Mycol. Soc. Trans. **33**: 113. 1950.

Ceratostomella piceae Münch, Naturw. Ztschr. f. Forst. u. Landw. **5**: 547. 1907.

Ophiostoma piceae (Münch) H. & P. Sydow, Ann. Mycol. **17**: 43. 1919.

Ceratostomella querci Georgew., Acad. des Sci. Compt. Rend., Paris, **183**: 759. 1926.

Perithecia superficial to partially immersed, the bases black, globose, 80–180 μ in diameter, unornamented or with a few ventral hairs up to 120 x 3.5 μ ; necks black, hyaline at the tip, up to 1000 μ long, 20–50 μ in diameter at the base and 5–25 μ at the tip; ostiolar hyphae hyaline, tapered, blunt at the tip, 15–25 in number, 10–25(–40) x 2–3 μ ; asci not seen; ascospores bean-shaped, 3–4.5 x 1.5–2 μ .

Cultures with a grayish white aerial mycelial mat becoming gray to brown in older colonies; abundant aerial mycelium in freshly isolated cultures, sometimes becoming suppressed or sparse in older strains; mycelial conidial formation abundant from the first; coremia appearing in 4–5 days in the center, eventually covering the whole colony, sometimes lacking in older strains; colonies often sectored, especially in older strains; undersurface of the agar unchanged in some isolates, usually brown to black; growth intermediate, 25 mm.; odor slightly acid. Aerial hyphae pale brown, frequently branched, thin-walled, septate, 1–3.5 μ in diameter, often growing together in strands of 2–10 filaments; submerged hyphae hyaline at first, becoming brown, branched, thin-walled, septate, interwoven, 4–5 μ in diameter, with scattered clavate to globose cells up to 8 μ in diameter. Mycelial conidia produced directly on the vegetative mycelium or on conidiophores hardly differentiated from the vegetative mycelium, hyaline to pale brown, thin-walled, septate, 10–25 x 1–2 μ , with conidia both of the *Cephalosporium* type and the *Cladosporium* type, hyaline, ellipsoid, often pointed at one end, 3–9 x 1–3 μ . Conidiophores of the *Graphium* type, the stalks dark brown to black, consisting of many closely agglutinated elongate cells, up to 1500 x 30 μ , flaring at the top, the head hyaline, globose, consisting of ramified hyphae imbedded in a drop of liquid up to 700 μ in diameter, with conidia hyaline, ellipsoid to ovoid, 3–5 x 1–2.5 μ (Plate 3, Fig. 1).

Illustrations: Münch, E. 1907. Naturw. Ztschr. f. Forst. u. Landw. **5**: 531–573, Figs. 5–15; Lagerberg, T., G. Lundberg, and E.

Melin. 1927. Sv. Skogsvårdsf. Tidskr. 25: 145-272, Figs. 9-12; Nisikado, Y. and K. Yamauti. 1935. Ohara Inst. f. Landw. Forsch. Ber. 6: 539-560, Plates XXV-XXIX; Siemaszko, W. 1939. Planta Polonica 7(3): 1-52, Plate III, Figs. 1-8; Davidson, R. W. 1953. Mycologia 45: 579-586, Fig. 1.

C. piceae was first described from Germany on spruce and fir (Münch, 1907; lectotype, BPI-FP 14395). Other European countries from which this species has been reported are Poland (Siemaszko, 1939), Russia (R.A.M. 10: 143), Finland (R.A.M. 18: 564), Sweden (Lagerberg, *et al.*, 1927; Mathiesen, 1950; culture JH-61 = Rg 7-52, AM-K), Norway (Robak, 1932), England (MacCallum, 1922; Bakshi, 1950; culture JH-65 = S-95c, FPRL-E), and Spain (R.A.M. 24: 392). This species is known in North America from Nova Scotia, Canada (culture JH-53 = 29122, FPRL-C) and from the western United States (Davidson, 1953; culture JH-54, from Davidson). It has also been reported from Japan (Nisikado and Yamauti, 1935). Although the species is commonly encountered on coniferous logs and lumber and less frequently on hardwoods, it is not a serious cause of stain as the mycelium in the wood produces a very light brown discoloration or none at all. *Ceratostomella querci* differs from *C. piceae* only in the color reaction to the substrate (Georgewitch, 1927) and is a synonym (culture JH-28 from CBS). No. 45, *Ceratostomella fagi* (Loos, 1932), described from beech in Germany seems to differ in the narrower ascospores and the smaller ostiolar hyphae, but may also be a synonym.

C. piceae has perithecia similar to the next three species, *C. cana*, *C. clavata*, and *C. floccosa*. *C. cana* has larger ascospores and has conidia which are globose. *C. clavata* has brown, spirally coiled ostiolar hyphae and has coremia which are club-shaped, rather than globose, at the top. *C. floccosa* has shorter ostiolar hyphae and the coremia are flat-topped. Cultures of *C. floccosa* also have a yellowish caste. Among species lacking the *Graphium* stage, careful comparison should be made with No. 29, *C. pilifera*, No. 30, *C. capillifera*, No. 31, *C. schrenkiana*, and No. 32, *C. ambrosia*.

23. CERATOCYSTIS CANA (Münch) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella cana Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 558. 1907.
Ophiostoma canum (Münch) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.

Perithecia superficial, the bases black, globose, 50-150 μ in diameter or "185 μ hoch und 156 μ breit" (Mathiesen, 1951), almost bare or ornamented with numerous black, thick-walled, septate, unbranched hyphae up to 300 x 2 μ ; necks black, slender, up to 1000 μ long, 20-25 μ in diameter at the base and 10-15 μ at the tip; ostiolar hyphae not seen, "mit zahlreichen gleichbreiten, stumpfen hyalinen Zilien von ungleicher Länge (Mittel 22 μ)" (Mathiesen); asci not seen; ascospores bean-shaped, slightly curved, 5-6 x 1.5-2.5 μ or "nierenförmig, 6.5 x 3.0 μ " (Mathiesen).

Cultures forming a white dense mat, becoming dark in the center in 4-6 days; abundant mycelial conidia formed after 24 hours; coremia appearing in the center in 6-8 days, soon scattered over the whole surface of the colony; undersurface of the agar brown to black; growth

intermediate, 30 mm.; odor not distinctive. Hyphae hyaline to brown, branched, thin-walled, septate, 1–5 μ in diameter. Conidiophores of two kinds, one the *Cephalosporium* type, simple, unbranched, hyaline, septate, up to 30 x 2 μ with conidia hyaline, subglobose to globose, up to 12 μ in diameter; the other the *Graphium* type, the stalks dark brown to black at the base, pale brown to hyaline at the tip, up to 800 x 25 μ , flaring out at the tip, the head globose, hyaline, consisting of ramified hyphae imbedded in a drop of liquid up to 1000 μ in diameter, with conidia hyaline, globose, up to 8 μ in diameter.

Illustrations: Münch, E. 1907. Naturw. Ztschr. f. Forst. u. Landw. 5: 531–573, Figs. 16 and 17; Mathiesen, Aino. 1950. Oikos 2: 275–308, Figs. 5–9.

C. cana was first described from Germany on pine. It also has been reported from Sweden associated with insect attacks on pine (Mathiesen, 1950, 1951; culture JH-58, AM-K). No type has been found for this species and it is not known if there is any preserved. Three collections sent to Hedgcock by Münch in 1909 are preserved (lectotype, BPI-2107; 2110, FP 14394, BPI). Zach (1929) suggested that *C. cana* is a variety of *C. piceae* and that the differences in the imperfect stages were due to environmental conditions. However, Mathiesen pointed out that *C. cana* is a distinct species with morphological differences in the perithecia as well as in the imperfect stages. *C. cana* had not been considered of great importance as a staining fungus, but Mathiesen reports it is one of the most abundant and injurious of the blueing fungi in Sweden.

C. cana differs from other species with a *Graphium* stage in the larger ascospores and the globose conidia.

24. ***Ceratocystis floccosa*** (Mathiesen) Hunt, comb. nov.

Ophiostoma floccosum Mathiesen, Sv. Bot. Tidskr. 45: 219. 1951.

Perithecia (from Mathiesen) superficial, the bases black, globose, 155 (110–182) μ in diameter, unornamented; necks black, often irregularly bent, 940–1600 μ long, 36–70 μ in diameter at the base and 12–20 μ at the tip; ostiolar hyphae hyaline, mostly 16 in number, 10–12.8 x 2.8 μ ; asci evanescent; ascospores kidney-shaped, 3.5 x 1.7 μ (3.3–4.7 x 1.5–2.6 μ).

Cultures white at first with abundant aerial mycelium, sometimes becoming appressed in older colonies; becoming light yellowish in a few days and finally golden brown to dark brown; often sectorial; scattered coremia appearing over the entire surface in about 1 week; perithecia not seen; growth intermediate, 30 mm.; odor not distinctive or "schwach honigartig" (Mathiesen). Aerial hyphae hyaline to pale brown, branched, septate, thin-walled, up to 5 μ in diameter; submerged hyphae similar except brown, up to 8 μ in diameter. Mycelial conidia produced individually or in whorls on short sterigmata on the vegetative mycelium with conidia hyaline, ovoid, some elongate, 5–8 x 2–4 μ , also on slightly differentiated conidiophores which are hyaline, septate, lateral branches 10–20 x 2–3 μ with conidia in clusters on short sterigmata at the tips and along the sides, hyaline, ellipsoid, often pointed at one end, 5–6 x 2.5–4 μ . Conidiophores of the *Graphium* type, the stalks pale brown, of agglutinated hyphae, flaring at the top,

up to $300 \times 40 \mu$ or "400–500 μ hoch und 50–60 μ breit" (Mathiesen), the heads hyaline, flat-topped or "diskusartig" (Mathiesen), consisting of ramified hyphae imbedded in a drop of liquid up to 300μ in diameter, with conidia hyaline, cylindrical to ellipsoid, $3\text{--}5 \times 1.5\text{--}2 \mu$.

Illustrations: Mathiesen, Aino. 1951. Sv. Bot. Tidskr. 45: 203–232, Figs. 3 and 4.

This species was found associated with bark beetles on pine and spruce in Sweden (Mathiesen, 1950, 1951; culture JH-59, AM-K). Perithecia of this species were not seen and the description of the perfect stages is taken entirely from Mathiesen (1951). No type has been designated but herbarium material and cultures are preserved at the Statens Skogsforskningsinstitut, Experimentalfältet, Sweden. *C. floccosa* is not of great importance as a staining fungus since it occurs only sporadically and is mixed with other species (Mathiesen, 1951).

C. floccosa differs from other species with *Graphium* stages by the short ostiolar hyphae. The coremia are light colored with a flat-topped appearance. The yellowish color of the cultures is also characteristic. Mathiesen (1951) has pointed out the similarity between the conidial stages of this species and of *Graphium silanum* Goid. (1936b, p. 246).

25. *Ceratocystis clavata* (Mathiesen) Hunt, comb. nov.

Ophiostoma clavatum Mathiesen, Sv. Bot. Tidskr. 45: 222. 1951.

Perithecia (from Mathiesen) often completely immersed in the wood, the bases brownish black, rather small, globose, somewhat flattened at the base, 166 ($127\text{--}212$) μ high and 177 ($127\text{--}238$) μ in diameter, unornamented; necks rather long, slender, somewhat tapered at the tip, 566 ($453\text{--}830$) μ long, 35.9 ($28.3\text{--}45.3$) μ in diameter at the base and 15.8 ($11.3\text{--}19.8$) μ at the tip; ostiolar hyphae always present, long, brown, characteristically spirally curved, $9\text{--}16$ (often 12) in number, 52.2 ($14\text{--}81.2$) μ long and 2.8μ wide; asci evanescent; ascospores long-elliptic in dorsal view, shaped like sections of an orange in side view, $3.3 \times 1.4 \mu$.

Cultures hyaline at first with an appressed mycelial mat, becoming dark olive-green to black in the center with a hyaline margin $5\text{--}6$ mm. wide, finally the whole colony becoming black; aerial hyphae sparse, a few scattered tufts and coremia produced after $1\text{--}2$ weeks; growth rapid, 40 mm.; odor not distinctive or "schwach aromatisch" (Mathiesen). Aerial hyphae sparse, mostly appressed, hyaline to pale brown, branched, thin-walled, septate, $1.5\text{--}3 \mu$ in diameter; submerged hyphae brown, densely interwoven, sometimes in strands composed of numerous hyphae, branched, thin-walled, septate, up to 5.5μ in diameter. Conidiophores rarely produced in culture, of two types, the one *Graphium*-like, with coremia somewhat club-shaped, the stalks pale to dark brown, tapered to the tip, up to $500 \times 50 \mu$, the heads somewhat elongate, with ramified branches along upper half of the stalk, the whole imbedded in a drop of liquid, with conidia hyaline, cylindrical to ellipsoid, $4\text{--}6 \times 2\text{--}2.5 \mu$; the other with short, scarcely differentiated side branches, hyaline, septate, unbranched, up to $35 \times 2 \mu$ with conidia as in the first type.

Illustrations: Mathiesen, Aino. 1951. Sv. Bot. Tidskr. 45: 203–232, Fig. 5.

This species was isolated in Sweden from the tops of fallen pines attacked by bark beetles (Mathiesen, 1950, 1951; culture JH-60, 8-1, AM-K). No perithecia of this species were obtained and the description of the perfect stages is taken entirely from Mathiesen (1951). Type specimens have not been designated, but herbarium material and cultures are preserved at the Statens Skogsforskningsinstitut, Experimentalfältet, Sweden.

This species is readily identified by the brown, spiral ostiolar hyphae, a character found in only one other species, No. 20, *C. brunneo-ciliata*. The shape of the ascospores differs in the two species; they are quadrangular in *C. brunneo-ciliata* and long-elliptic or shaped like sections of an orange in *C. clavata*.

26. CERATOCYSTIS ULMI (Buisman) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella ulmi Buisman, Tijdschr. over Plantenziekten 38: 1. 1932.

Ophiostoma ulmi (Buisman) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408. 1934.

Perithecia superficial to partially immersed, the bases black, globose, up to $135\ \mu$ in diameter; ornamented with short, black, septate hyphae up to $80 \times 3\ \mu$; necks black, slender, $180\text{--}360\ \mu$ long, $19\text{--}24\ \mu$ in diameter at the base and $11\text{--}16\ \mu$ at the tip; ostiolar hyphae numerous, hyaline, septate, thin-walled, the tips occasionally swollen, up to $30 \times 2\ \mu$, the swollen tips up to $5\ \mu$ in diameter, "cilien, ≈ 24 in aantal en van zeer verschillende lengte (gewoonlijk $25\text{--}26\ \mu$) . . . gesepteerd en dikwijls aan de top opgezwollen" (Buisman); asci not seen; ascospores crescent-shaped to shaped like the sections of an orange, $4.5\text{--}6 \times 1.5\ \mu$.

Cultures pale yellow to white with an abundant, loosely interwoven, stringy, somewhat radially aligned aerial mycelium; with concentric zones if grown in intermittent light; sometimes becoming grayish to black with the development of coremia; abundant mycelial conidia produced after 24-36 hours, the whole aerial mycelium becoming covered with conidia; perithecia and coremia rarely developed on agar; growth rapid, 45 mm.; odor not distinctive. Aerial hyphae hyaline, some becoming pale brown, branched, thin-walled, septate, $1\text{--}6\ \mu$ in diameter, some filaments growing together into strands of 2-10 hyphae; submerged hyphae similar, up to $8\ \mu$ in diameter. Conidiophores of two types, the one Cladosporium-like, but slightly differentiated from the vegetative mycelium, thin-walled, septate, with short sterigmata on which the spores are borne, the conidia hyaline, usually elongate-ellipsoid but varying in shape, $4.5\text{--}14 \times 2\text{--}3\ \mu$; the other of the *Graphium* type, the stalks black, slender, composed of a few to many agglutinated hyphae, up to several mm. tall and up to $50\ \mu$ in diameter, usually not exceeding $1000 \times 20\ \mu$, flared at the tip, the heads hyaline, globose, composed of hyaline ramified branches imbedded in a drop of liquid, up to $200\ \mu$ in diameter, with conidia hyaline, ellipsoid, $4.5\text{--}5.5 \times 1\text{--}1.5\ \mu$.

Illustrations: Buisman, C. 1932. Tijdschr. over Plantenziekten 38: 1-5, Plates I-III.

C. ulmi is the perfect stage of *Graphium ulmi* Schwartz (1922). Perithecia were first described by Buisman from a culture on sterilized elm twigs (isotype, from J. A. von Arx, deposited at the Phytopatho-

logischen Laboratorium "Willie Commelin Scholten," Baarn, Netherlands). In addition to the isotype, perithecial material was obtained from Roger V. Swingle, USDA Horticultural Crops Research Branch, Worthington, Ohio (146, SYRF), and cultures were studied from New York (JH-2), American Type Culture Collection 9350 (JH-21), and CBS (JH-27). In nature the fungus produces coremia in the tunnels of bark beetles and in cracks in the bark and wood. Perithecia are rarely found in nature. *C. ulmi* is the causal organism of the destructive Dutch elm disease (Boyce, 1948), which has been found both in Europe and North America on most species of elm (for its distribution see Imperial Mycological Institute, Distribution Maps of Plant Diseases, No. 36, 2nd Edition, 1947).

This fungus differs from other species with a *Graphium* stage in having small perithecia with short necks.

Section 3. Species with mycelial conidia only
(*Ophiostoma* group in part)

I. *Perithecia with necks more than 500 μ long*

A. *Ostiolar hyphae present*

B. *Necks with several whorls or annuli*

27. CERATOCYSTIS PLURIANNULATA (Hedgc.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella pluriannulata Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 72. 1906.

Ophiostoma pluriannulatum (Hedgc.) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.

Perithecia superficial, the bases black, globose, 80–250 μ in diameter; unornamented or covered with hyaline to pale brown undifferentiated hyphae or sometimes with dark brown, thick-walled, slightly tapered hyphae up to 25 x 3 μ ; necks black, up to 3000 μ long, 20–40 μ in diameter at the base and 7–15 μ at the tip; ostiolar hyphae hyaline, more than 20 in number, tapered, up to 60 μ long, 2.5 μ in diameter at the base and 1 μ at the tip; 3–6 annuli present at irregular intervals along the neck, sometimes without hyphae or with 1–20 hyphae similar to those at the tip; asci not seen; ascospores bean-shaped or shaped like sections of an orange, 3.5–5 x 1–2 μ .

Cultures white at first with abundant aerial mycelium, remaining almost pure white; often becoming tufted; conidia produced in 1 day; numerous perithecia appearing in 1–2 days and maturing in 4–5 days in freshly isolated strains; in older strains perithecia appearing in 2–4 weeks or often lacking; undersurface of the agar unchanged or becoming pale brown or black in some isolates; often sectoried; growth intermediate, 20 mm.; odor not distinctive. Aerial hyphae hyaline to pale brown, frequently branched, thin-walled, septate, 1–5.5 μ in diameter; submerged hyphae similar but 3–8 μ in diameter. Conidiophores hyaline, simple to branched, septate, making up most of the aerial mycelium, frequently grown together to form tree-like tufts, up to 150 x 1.5 μ , with conidia of the *Cladosporium* type, hyaline, elongate-ellipsoid, tapered to a point at one end, 5–7.5 x 1.5–2.5 μ (Plate 3, Fig. 2).

Illustrations: Hedgcock, G. G. 1906. Mo. Bot. Gard. Ann. Rpt. 17: 59–114, Plate 3, Fig. 7 and Plate 5, Figs. 1, 2; Lagerberg, T.,

G. Lundberg, and E. Melin. 1927. Sv. Skogsvårdsf. Tidskr. 25: 145-272, Figs. 13-15; Gregor, M. J. F. 1932. Ann. Mycol. 30: 1-9, Figs. 1-4.

C. pluriannulata was described from oak in Indiana (TYPE, BPI; isotype, FH). Specimens have been examined from North Carolina, Virginia, Maryland (BPI, FH, NY), and New York (culture JH-16 = 2, R. A. Zabel), and it has been reported from the South (Davidson, 1935; Verrall, 1939) on both coniferous lumber and hardwoods, but much more commonly on the latter. The species has also been found in British Columbia, Canada (culture JH-52 = 29121, FPRL-C), and in Europe *C. pluriannulata* is reported in Sweden (Lagerberg *et al.*, 1927) and in England (Gregor, 1932). The fungus causes a light discoloration which is not deeply penetrating on both hardwood and coniferous wood, consequently, even though common, it is not one of the important staining fungi.

C. pluriannulata is readily identified by the pure white cultures and the long-necked perithecia with several whorls or annuli. Compare No. 50, *Ceratostomella stenoceras*, an imperfectly known species.

28. ***Ceratocystis multiannulata*** (Hedgc. & Davids.)

Hunt, comb. nov.

Ceratostomella multiannulata Hedgc. & Davids., Jour. Agr. Res. 50: 797. 1935.

Ophiostoma multiannulatum (Hedgc. & Davids.) von Arx, Antonie van Leeuwenhoek 18: 211. 1952.

Perithecia superficial, the bases black, globose, 120-250 μ in diameter, ornamented with short, black, globular protuberances 6-15 μ in diameter (Plate 4, Fig. 2); necks black, long, slender, with 3-7 annuli scattered at irregular intervals, up to 9000 μ long, 25-40 μ in diameter at the base and 10-15 μ at the tip; ostiolar hyphae hyaline, short, blunt at the tips, up to 11 x 2 μ ; asci not seen; ascospores extruded in droplets along the neck and at the tip, bean-shaped, 3.5-4.5 x 1.5-2 μ (Plate 1, Fig. 8).

Cultures hyaline at first, turning dark in 2-3 days; often sectoried; conidia appearing in 1-2 days; perithecia appearing in 1 week, maturing in 2-3 days; undersurface of the agar turning brown to black; growth intermediate, 30 mm.; odor not distinctive. Aerial hyphae hyaline, rarely to frequently branched, thin-walled, 1-1.5 μ in diameter; submerged hyphae pale brown, frequently branched, some cells swollen, interwoven, thin- to moderately thick-walled, frequently septate, 2-5.5 μ in diameter. Conidiophores simple, hyaline, septate hyphae often grown together to form a tree-like tuft, up to 50 x 3 μ , with conidia produced in clusters at the tips and occasionally along the sides of the conidiophores, the conidia hyaline, long and slender, tapered at one end, 5-13 x 1-2.5 μ or "borne at enlarged denticulate apex in groups of 5 to 10, hyaline, one-celled, pointed at one end, 2 μ to 3.2 μ by 6 μ to 25 μ " (Davidson).

Illustrations: Davidson, R. W. 1935. Jour. Agr. Res. 50: 789-807, Fig. 1A-D.

This fungus was isolated from pine lumber by Davidson (TYPE, BPI-FP 59039, JH-4 from CBS). It has also been found in Maryland (BPI), and Georgia (Verrall, 1939). The species is one of the fungi

most commonly encountered on freshly sawn pine lumber in the South, but is of doubtful importance as a staining fungus (Davidson, 1935).

The preceding species, *C. pluriannulata*, differs from *C. multiannulata* in the fewer annuli on the neck and in the absence of globose protuberances on the base of the perithecium. No. 31, *C. schrenkiana*, is similar but lacks the additional annuli on the neck and the globose protuberances on the base of the perithecium.

BB. Necks with only a terminal whorl of ostiolar hyphae

29. CERATOCYSTIS PILIFERA (Fries) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Sphaeria pilifera Fries, Syst. Mycol. 2: 472. 1822.

Ceratostoma piliferum (Fries) Fuckel, Symb. Mycol., p. 128. 1869.

Ceratostomella pilifera (Fries) Winter, Rabenh. Kryptogamen-Flora 1: 252. 1887.

Linostoma piliferum (Fries) von Höhnelt, Ann. Mycol. 16: 91. 1918.

Ophiostoma piliferum (Fries) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.

Ceratostomella coerulea Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 561. 1907.

Ceratostomella echinella Ell. & Ev. emend. Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 69. 1906.

Perithecia superficial to partially immersed, the bases black, globose, sometimes flattened on the bottom, 100–250 μ in diameter, unornamented or with brown, thin-walled, septate, ventral hyphae 30–200 \times 2–4 μ , or with undifferentiated hyphae attached to all sides; necks black, slender, up to 2000 (–3000) μ long, 20–40 μ in diameter at the base and 9–16 μ at the tip; ostiolar hyphae hyaline, 10–20 in number, spreading, blunt at the tips, not tapered or very slightly tapered, 20–65 \times 2–2.5 μ ; asci not seen; ascospores bean-shaped to shaped like sections of an orange, 4–6 \times 1–2 μ (Plate 1, Fig. 7).

Cultures white at first with cottony aerial mycelium, remaining white in some isolates, becoming dense black in others, with a few scattered patches of white; aerial hyphae abundant, floccose, sometimes becoming appressed; great quantities of conidia produced after 1 day; perithecia appearing in 4–6 days, maturing in 5–7 days; under-surface of the agar black; growth intermediate, 30 mm.; odor not distinctive or slightly acid. Aerial hyphae hyaline to pale brown, frequently branched, thin-walled, septate, 2–3 μ in diameter, sometimes several filaments growing together to form strands; submerged hyphae hyaline at first, quickly becoming brown to almost black, interwoven, frequently branched, thin-walled, septate, with some cells swollen and almost globose, 2–8 μ in diameter. Conidiophores scarcely differentiated from the vegetative mycelium, hyaline, sometimes branched, thin-walled, septate, up to 35 \times 2 μ ; with conidia of two types, some hyaline, cylindrical to elongate-ellipsoid, usually pointed at the end of attachment, Cladosporium-like, 6–12 \times 2–4 μ , others hyaline, ellipsoid, pointed at the end of attachment, 5–6 \times 2.5–3.5 μ and borne on short sterigmata in clusters along the sides of and at the tips of the conidiophores (Plate 3, Fig. 3).

Illustrations: (as *Ceratostomella pilifera*) von Schrenk, H. 1903. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 36: 1–39, Plate VII, Figs. 1–9; Hedgcock, G. G. 1906. Mo. Bot. Gard. Ann. Rpt. 17: 59–114, Plate 3, Fig. 8, and Plate 4, Figs. 5–7; Goidanich, G. 1936. R.

Staz. Pat. Veg. Bol., Rome, n.s. 16: 225–270, Figs. 1–8; Siemaszko, W. 1939. *Planta polonica* 7(3): 1–52, Plate I, Figs. 6–8, and Fig. 1C; (as *Ceratostomella coerulea*) Münch, E. 1907. *Naturw. Ztschr.f. Forst. u. Landw.* 5: 531–573, Figs. 1, 18, and 19; Lagerberg, T., G. Lundberg, and E. Melin. 1927. *Sv. Skogsvårdsf. Tidskr.* 25: 145–272, Figs. 1–8.

During the nineteenth century the specific name "*pilifera*" was applied to a group of similar species. Hedgcock (1906) added to the description of *Ceratostomella pilifera* given by von Schrenk (1903) and designated one of von Schrenk's collections as the lectotype (BPI). Münch (1907) recognized *Ceratostomella pilifera* as a group of species, the "*Pilifera-Gruppe*." One of these he described as *Ceratostomella coerulea*. Specimens of *C. coerulea* sent to Hedgcock by Münch in 1909 (BPI, NY) are identical with *C. pilifera* in the sense of Hedgcock. *C. coerulea* is a synonym of *C. pilifera* as has already been indicated by Goidanich (1936b) and Siemaszko (1939). *Linostoma piliferum* was designated as the type for the genus *Linostoma* (von Höhnelt, 1918). *Ceratostomella echinella* Ell. and Ev. as emended by Hedgcock is a synonym (BPI), but *C. echinella* Ell. and Ev. is quite different and is an excluded species (which see).

Ceratocystis pilifera is widespread on coniferous lumber in the United States (BPI, FH, MO, NY). Davidson (1935) and Verrall (1939) report it as one of the most important of the staining fungi in the South. In Europe the species has been found in Germany (BPI, NY), Poland (Siemaszko, 1939), Russia (R.A.M. 10: 143), Latvia (R.A.M. 19: 181), Finland (R.A.M. 18: 564), Sweden (Lagerberg *et al.*, 1927; Melin and Nannfeldt, 1934; and Mathiesen, 1950), Belgium (R.A.M. 26: 267), Spain (R.A.M. 24: 392), and Italy (Goidanich, 1936b). It has also been reported from Australia (R.A.M. 13: 341). *C. pilifera* causes a deeply penetrating dark blueish discoloration in the sapwood.

The species differs from No. 27, *C. pluriannulata*, which has additional whorls or annuli on the neck, and from the next species, *C. capillifera*, which has ostiolar hyphae that taper to an attenuated point.

30. *CERATOCYSTIS CAPILLIFERA* (Hedgc.) C. Moreau, *Revue de Mycologie, Suppl. Colonial* 17: 22. 1952.

Ceratostomella capillifera Hedgc., *Mo. Bot. Gard. Ann. Rpt.* 17: 71. 1906.

Ophiostoma capilliferum (Hedgc.) H. & P. Sydow, *Ann. Mycol.* 17: 43. 1919.

Ceratocystis longirostellata Bakshi, *Commonw. Mycol. Inst., Mycol. Paper* 35: 8. 1951.

Perithecia superficial, the bases black, globose, sometimes somewhat flattened ventrally, 75–200 μ in diameter, unornamented or with undifferentiated hyphae attached on all sides; necks black, slender, 600–3000 μ long, 15–40 μ in diameter at the base and 5–21 μ at the tip; ostiolar hyphae hyaline, numerous, tapered to a long attenuated tip, thin-walled, septate, 40–100 μ long and 2–3 μ in diameter at the base; asci not seen; ascospores shaped like sections of an orange, slightly curved, 3–5.5 x 1–2.5 μ .

Cultures white at first with abundant aerial mycelium which remains white; substrate hyphae quickly becoming black; numerous

conidia produced after 1-2 days; perithecia appearing in 2-3 days, maturing in 1-2 weeks; undersurface of the agar black, growth intermediate, 30 mm.; odor slightly acid. Aerial hyphae hyaline to pale brown, rarely to frequently branched, thin-walled, frequently septate, $1.5-3(-5) \mu$ in diameter; substrate hyphae similar except brown to black and $3-8 \mu$ in diameter. Conidiophores scarcely differentiated from the vegetative mycelium, hyaline, thin-walled, septate, $1-2 \mu$ in diameter, with conidia hyaline, cylindrical, pointed at the end of the attachment, borne in clusters on short sterigmata at the ends and along the sides of the conidiophores, $5-7.5 \times 1-3 \mu$.

Illustrations: (as *capillifera*) Hedgcock, G. G. 1906. Mo. Bot. Gard. Ann. Rpt. 17: 59-114, Plate 3, Fig. 1, and Plate 6, Fig. 2; (as *longirostellata*) Bakshi, B. K. 1951. Commonw. Mycol. Inst., Mycol. Paper 35: 1-16, Plate III, Figs. 15-18, and Fig. 3.

C. capillifera was described from sweet gum in Arkansas (TYPE, BPI-FP 46618; isotype, FH). The species has subsequently been found on a number of different genera of hardwoods and on pine in Maryland, North Carolina, Tennessee, and Illinois (BPI, NY). *C. longirostellata*, described from oak in Scotland, is a synonym (IMI-20165; culture JH-66 = S-98, FPRL-E). This fungus causes a dark gray to black discoloration of wood.

The preceding species, *C. pilifera*, is similar to *C. capillifera* except that the ostiolar hyphae are not attenuated. No. 27, *C. pluriannulata*, differs in the additional annuli along the neck.

31. CERATOCYSTIS SCHRENKIANA (Hedgc.) C. Moreau, Revue de Mycologie, Suppl. Colonial 17: 22. 1952.

Ceratostomella schrenkiana Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 67. 1906.

Ophiostoma schrenkianum (Hedgc.) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.

Perithecia superficial, the bases black, globose, up to 140μ in diameter or " 120μ to 200μ " (Hedgcock), unornamented or with black, thick-walled, septate hyphae up to $100 \times 3 \mu$; necks black, slender, hyaline at the tip, up to 1000μ long, $20-30 \mu$ in diameter at the base and $10-20 \mu$ at the tip; ostiolar hyphae not seen, "a row of short, hyaline, spreading bristles, . . . about 10μ to 15μ by 2μ " (Hedgcock); asci not seen; ascospores bean-shaped, $3.5-4.5 \times 1-2.5 \mu$ or " 2.5μ to 4μ by 1μ to 1.5μ " (Hedgcock).

In culture "filaments and hyphae remain white for several days, and often become massed in a furry outgrowth in which strands of filaments unite into upright clusters or false heads. After a few days' growth portions of the mycelium lying next the agar become pigmented with a brown color, and rapidly develop perithecia" (Hedgcock). Hyphae hyaline to brown, " 3μ to 7μ in diameter" (Hedgcock). Conidiophores hyaline, thin-walled, often clustered together into a tree-like tuft, with conidia borne in clusters on short sterigmata at the tips, the conidia hyaline, cylindrical, often tapered to a point at the end of the attachment, $5-7 \times 1-1.5 \mu$. "In nature, the perithecia that mature are often surrounded by several closely adhering dark bodies, which are either sclerotia or abortive perithecia, apparently the latter" (Hedgcock).

Illustrations: Hedgecock, G. G. 1906. Mo. Bot. Gard. Ann. Rpt. 17: 59–114, Plate 3, fig. 6, and Plate 4, figs. 1–4.

C. schrenkiana was described from pine in Missouri (TYPE, BPI, isotypes, FH, NY), and is known only from the type locality. It produces a blue-black stain in wood.

This species is similar in perithecial morphology to No. 29, *C. pilifera*, but varies from the latter in the shorter ostiolar hyphae and the smaller ascospores. No. 42, *Ceratostomella castaneae*, also appears to be similar, but differs in the bacilliform ascospores. The dendroid tufts of conidiophores are characteristic of Nos. 27 and 28, *C. pluriannulata* and *C. multiannulata*, but this species lacks the additional whorls or annuli. Some of the specimens of Hedgecock labeled *C. schrenkiana* also have perithecia of *C. multiannulata*.

32. CERATOCYSTIS AMBROSIA Bakshi, Brit. Mycol. Soc. Trans. **33**: 116. 1950.

Perithecia superficial to partially immersed, the bases black, globose, 150–200 μ in diameter or “109 to 221 μ and 103 to 214 μ (average 165 and 157 μ)” (Bakshi), ornamented with a few brown, moderately thick-walled hyphal hairs 10–50 \times 3–4 μ and with undifferentiated hyphae; necks black, sometimes bent, 420–480 μ long or “240 to 924 μ ” (Bakshi), 35–50 μ in diameter at the base and 11–16 μ at the tip; ostiolar hyphae not seen, they “may be absent at the ostiole or may be present, the maximum count made being ten. They vary in length from 21 to 25 μ , though occasionally cilia 10 μ long may be seen, and are mostly 1-celled, rarely 2–3-celled by cross-walls, thin-walled, hyaline, 1.7–2.3 μ broad at the base, tapering to 0.8–1.2 μ at the tip” (Bakshi); asci not seen; ascospores bean-shaped, 3–5 \times 1.5–2 μ .

In culture “the growth is appressed and sodden with very scanty aerial mycelium . . . mycelium is mostly colourless in Petri dishes, while in tubes pigmentation appears . . . conidia begin to appear in about a week . . . perithecia begin to appear in cultures about two weeks old and they mature after about three weeks . . . the rate of radial growth is about 3.5 cm. in 10 days” (Bakshi). Hyphae hyaline to brown, branched, thin-walled, some becoming thick-walled, septate, 2–6 μ in diameter. Conidiophores hyaline, thin-walled, with short side branches up to 30 \times 2 μ , the conidia hyaline, ellipsoid to ovate, 3–12 \times 2–4 μ , borne in clusters at the tips and along the sides of the conidiophores. “Sclerotia . . . produced abundantly in culture . . . are round, dark-brown to black bodies . . . 50–65 μ in diameter” (Bakshi).

Illustrations: Bakshi, B. K. 1950. Brit. Mycol. Soc. Trans. **33**: 111–120, Plate XI, Figs. 20–24, and Fig. 2.

C. ambrosia was described from cultures isolated from birch infested with ambrosia beetles in Scotland (lectotype, IMI-20166; dried culture S-96 from FPRL-E, SYRF).

The species is similar to the preceding species, *C. schrenkiana*, which differs in the shorter ostiolar hyphae. Cultures of *C. ambrosia* have little or no aerial mycelium; those of *C. schrenkiana* have abundant aerial mycelium.

33. *Ceratocystis tetropii* (Mathiesen) Hunt, comb. nov.

Ophiostoma tetropii Mathiesen, Sv. Bot. Tidskr. **45**: 228. 1951.

Perithecia superficial to partially immersed, the bases black, globose, up to 250 μ in diameter, ornamented with brown, unbranched, thin-walled, septate hyphal hairs up to 200 x 3 μ ; necks black, pale brown to hyaline at the tip, up to 1000 μ long, 35–55 μ in diameter at the base and 16–22 μ at the tip; ostiolar hyphae pale brown at the base, hyaline at the tip, tapered to a point, septate at the base, thin-walled, up to 40 μ long and 3.5 μ in diameter at the base; asci not seen; ascospores short cylindrical, slightly curved, blunt at the ends, 3–4 x 1–1.5 μ (Plate 1, Fig. 10).

Cultures with abundant dull white aerial mycelium, becoming pale yellow-brown, loosely interwoven, covered with droplets containing conidia; perithecia appearing in 6–10 days, maturing in 2–3 weeks; growth rapid, 45 mm.; odor not distinctive. Hyphae pale brown, branched, thin-walled, septate, 1–6 μ in diameter. Conidiophores of two types, the one *Cephalosporium*-like, hyaline, simple, thin-walled, with 2–3 nonseptate side branches often joined together at the tips, up to 25 x 1.5 μ , with the conidia produced at the tips hyaline, cylindrical to ovoid, rounded at both ends or pointed at one end, 3–5 x 1.5–2 μ (Plate 3, Fig. 5); the other resembling a *Leptographium*, the stalks short, hyaline to pale brown, 2–3 celled, 10–30 x 3–5 μ , with loose ramified heads, up to 50 μ in diameter, the whole structure 60–120 μ tall, with conidia as in the *Cephalosporium* type.

Illustrations: Mathiesen, Aino. 1951. Sv. Bot. Tidskr. **45**: 203–232, Figs. 6 and 7.

C. tetropii was found in Sweden associated with bark beetles on standing dead and half-dead spruce (1–3, 8–2, AM-K; culture JH-76 = 231–52, AM-K). As a blueing fungus it is not of great importance, as it only occurs in the wood adjacent to the tunnels of the insect larvae and does not produce a dark discoloration (Mathiesen, 1951).

This species differs from all other species of the group with mycelial conidia in having sharply pointed erect ostiolar hyphae, short cylindrical ascospores, and long, brown, hyphal hairs on the base of the perithecium. No. 47, *Ceratostomella lignorum*, an imperfectly known species, appears to be similar.

AA. Ostiolar hyphae absent**34. *Ceratocystis montia* (Rumb.) Hunt, comb. nov.**

Ceratostomella montium Rumb., Jour. Agr. Res. **62**: 597. 1941.

Ophiostoma montium (Rumb.) von Arx, Antonie van Leeuwenhoek **18**: 211. 1952.

Perithecia superficial to immersed, the bases black, appearing brown under the microscope in younger specimens, globose, 180–440 μ in diameter, unornamented or covered with a thick mat of differentiated hyphae; necks black, pale brown to hyaline at the apex, often swollen near the base, 1000–2000 (–4000) μ long, 40–100 μ in diameter at the base and 10–30 μ at the tip; ostiolar hyphae absent; asci not seen; ascospores with gelatinous sheath in the form of flanges, rectangular, “rectangular parallelepipedal with square ends” (Rumbold), 3–5 x 2–3 μ .

In culture "young colonies with conidia white, changing to warm sepia and black" (Rumbold). Hyphae hyaline to brown, branched, thin-walled, septate, interwoven and forming a dense mat, some cells swollen, 2–6 μ in diameter. Conidiophores simple, hyaline, bearing spores in clusters at the tips, up to 1 μ in diameter, with conidia hyaline, cylindrical to ellipsoid, 2–3 x 1–1.5 μ or "conidiophores single, hyaline, at first unbranched, later [developing into] simple branched hyphae bearing conidia in clusters; conidia hyaline, clustered or solitary, appearing on hyphae and short conidiophores, globular, 4 μ to 5 μ , ovoid to clavate, 6.5 μ to 8 μ by 4 μ to 5 μ " (Rumbold).

Illustrations: Rumbold, C. T. 1941. Jour. Agr. Res. 62: 589–601, Figs. 2–5; Taylor-Vinje, M. 1940. Mycologia 32: 760–775, Figs. 1–30.

C. montia was found associated with the bark beetles *Dendroctonus monticolae* and *D. ponderosae* on various species of pines in the states of Washington, Idaho, Utah, Colorado, Wyoming, and South Dakota (lectotype, BPI-606 CTR; also numerous collections, BPI, NY). The fungus stains the wood a gray color, beginning its growth in the galleries and penetrating the inner bark and the sapwood. It grows in toward the heartwood, staining the sapwood (Rumbold, 1941).

This species differs from other species with mycelial conidia in the shape of the ascospores and in the absence of ostiolar hyphae. It is similar to *C. ips* which differs in having smaller perithecial bases.

35. *Ceratocystis perparvispora* Hunt, nomen nov.

Ceratostomella (*Ophiostoma*) *microspora* Davids., Mycologia 34: 650. 1942, not *C. microspora* Ell. & Ev. (1893).

Ophiostoma microsporum (Davids.) von Arx, Antonie van Leeuwenhoek 18: 211. 1952.

"Perithecia . . . black, nearly spherical, 200–270 μ in diameter, thick walled; beaks [necks] occasionally two on a perithecium, 1.2–1.6 mm. long by 60–75 μ thick at the base to 18–19 μ thick just below ostiole; no filaments around the ostiole, but in very mature condition hyphae spread slightly to form a funnel-like opening; asci elongate ovoid, small, evanescent; ascospores not collecting in a globule at the ostiole but running down on the outside of the beak, light pinkish-brown in mass, hyaline under the microscope, minute, 1.5–2.5 x 0.5 μ " (Davidson).

Cultures white from the first and remaining so, with radial streaks; "a dense white growth of aerial mycelium and conidiophores covers the surface of the substratum and usually persists even after the perithecia are mature . . . perithecia begin to form in about two weeks, maturing slowly" (Davidson); growth slow, 8 mm., or "5 mm. in 5 days" (Davidson); odor slightly acid. Hyphae hyaline, branched, thin-walled, septate, up to 3 μ in diameter. Conidiophores scarcely differentiated from the vegetative mycelium, hyaline, septate, up to 25 x 2 μ , with conidia borne directly on the vegetative mycelium or in a Cephalosporium-like manner at the apex of the conidiophore, the conidia hyaline, ellipsoid, often curved and some almost sickle-shaped, 3.5–10 x 1.5–2.0 μ or "usually slightly curved, 4–10 x 1.2–2 μ , hyaline" (Davidson).

Illustrations: (as *microspora*) Davidson, R. W. 1942. *Mycologia* 34: 650-662, Fig. 1H-K, and Fig. 2G-I.

C. perparvispora was isolated from a chestnut stump in Pennsylvania, and from the heartwood of oak in Virginia. It is known only from a single culture which is apparently atypical since little or no aerial mycelium is produced (JH-76, BPI). No type was designated and no herbarium material could be found. The species is not known to cause pronounced discoloration or disease in the host substrate (Davidson, 1942).

C. perparvispora differs from other species of the mycelial conidial group in the shape and size of the ascospores and the absence of ostiolar hyphae.

II. *Perithecia with necks less than 500 μ long*

36. *Ceratocystis minor* (Hedgc.) Hunt, comb. nov.

Ceratostomella minor Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 74. 1906.

Ophiostoma minus (Hedgc.) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.

Ceratostomella exigua Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 76. 1906.

Ceratostomella pini Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 541. 1907.

Ceratostomella pseudotsugae Rumb., Jour. Agr. Res. 52: 431. 1936.

Perithecia superficial to immersed, the bases black, globose, up to 100 (-120) μ in diameter, unornamented or with a few dark brown to black, thick-walled, septate hyphae attached ventrally, up to 5 μ in diameter; necks black, short, often bent, up to 150 μ long but usually not exceeding the diameter of the base, 12-20 μ in diameter at the base and 6-15 μ at the tip; ostiolar hyphae sometimes lacking, when present hyaline, blunt at the tips, variable in number, usually 6-10, and in size, 6-19 x 1.5 μ ; asci not seen; ascospores crescent- to sickle-shaped, slightly tapered at each end, 4-5.5 x 1-1.5 μ (Plate 1, Fig. 6).

Cultures white at first, with sparse to abundant aerial mycelium, soon becoming appressed in most isolates; colonies turning black in 2-3 days in some isolates, remaining white to gray in others or turning black after 1-2 weeks; conidia produced from the first; perithecia appearing in 1-2 weeks; in some isolates numerous black sclerotia develop on the surface of the agar, in others sclerotia lacking; under-surface of the agar black in some isolates, remaining unchanged in others; growth rapid, 40 mm.; odor slightly acid. Aerial hyphae hyaline at first, becoming pale brown, frequently branched, thin-walled, septate, 1-4 μ in diameter; submerged hyphae dark brown to black, often covered with amorphous material, interwoven, frequently branched, septate, very irregular in shape and size, 2-12 μ in diameter. Conidiophores short side branches, hyaline, up to 25 x 2 μ , with conidia hyaline, ellipsoid to ovoid, somewhat truncate or rounded at the ends, 3.5-6 x 2-3.5 μ ; conidiophores sometimes arising from a brown stalk and forming a brushlike mass of ramified hyphae somewhat resembling a *Leptographium*. Sclerotia, when present in culture, black, globose or irregularly shaped, sometimes with imbedded perithecia, up to 3 mm. in diameter, composed of numerous, closely packed, thin-walled, pseudoparenchymatous cells up to 20 μ in diameter; in nature sclerotia usually occurring between the bark and the wood, black, pillarlike structures, up to 4 mm. long.

Illustrations: (as *Ceratostomella minor*) Hedgcock, G. G. 1906. Mo. Bot. Gard. Ann. Rpt. 17: 59-114, Plate 3, Fig. 4, and Plate 5, Fig. 6, (Fig. 7 does not agree with spores found on type); (as *Ceratostomella pini*) Münch, E. 1907. Naturw. Ztschr. f. Forst. u. Landw. 5: 531-573, Figs. 2-4; Lagerberg, T., G. Lundberg, and E. Melin. 1927. Sv. Skogsvårdsf. Tidskr. 25: 145-272, Figs. 16-21; Nisikado, Y., and K. Yamauti. 1934. Ohara Inst. f. Landw. Forsch. Ber. 6: 467-490, Plates 17, 19-21.

C. minor was first described from pine in Arizona (TYPE, BPI; isotypes FH, NY). The original description is incomplete and the ascospores found on the type do not agree with the illustration nor with the description, "3.1 μ to 4.2 μ in length and .9 μ to 1.9 μ in width, averaging 3.5 μ by 1.5 μ " (Hedgcock). Some collections of *C. minor* are annotated with "Type collection has sclerotia under the bark. I consider *C. pini* Münch a synonym. G. G. Hedgcock." Comparison of authentic material of the two species reveals that *Ceratostomella pini* and *C. minor* are identical. The shape and the size of the ascospores of *C. exigua* as given by Hedgcock in the original description do not agree with those found on the type (BPI). *Ceratostomella pseudotsugae* differs only in the absence of sclerotia and in physiological reactions in cultures. Sclerotia are not always produced by *C. minor* and as different isolates vary greatly in culture, *C. pseudotsugae* is considered a synonym of *C. minor*. *C. minor* has been reported (under the name *C. pini*) from several European countries including Germany (Münch, 1907, BPI), Poland (Siemaszko, 1939), Russia (R.A.M. 10: 143), Sweden (Lagerberg *et al.*, 1927), England (MacCallum, 1922), and Spain (R.A.M. 24: 392). It is widespread in the United States (Rumbold, 1931; BPI, FH, NY) and has also been recorded in Japan (Nisikado and Yamauti, 1934). This species causes a dark gray to almost black stain in wood and is considered one of the most important blueing fungi in Sweden (Lagerberg *et al.*, 1927). *C. minor* has not been shown to be an important cause of stain in the United States. It is commonly found associated with various bark beetles on coniferous hosts and causes the death of a tree by cutting off the water supply to the crown (Nelson and Beal, 1929; Bramble and Holst, 1940).

C. minor is readily identified by the small short-necked perithecia and the crescent-shaped ascospores. The species is variable in culture, some isolates producing both perithecia and sclerotia in abundance, others perithecia only, and others conidial stages only.

✓ 37. *Ceratocystis albida* (Mathiesen-K.) Hunt, comb. nov.

Ophiostoma albidum Mathiesen-K., Meddel. Statens Skogsforskningsinst., Sweden, 43(4): 50. 1953.

Perithecia superficial to immersed, the bases black, globose, up to 95 μ in diameter in culture, up to 140 μ on wood, unornamented or with a few undifferentiated, brown hyphae; necks black, relatively stout, up to 400 μ long or "340-480 (423) μ " (Mathiesen-Käärik), 11-27 μ in diameter at the base and 8-11 μ at the tip; ostiolar hyphae sometimes absent, hyaline, spreading, 10-15 in number, up to 20 x 1.5 μ ; asci not seen; ascospores bean-shaped or cylindrical and slightly curved, 3.5-4 x 1-1.5 μ .

Cultures hyaline to white with little or no aerial mycelium; perithecia appearing after 4-6 weeks, maturing slowly; undersurface of the agar unchanged; growth slow, 8 mm.; odor not distinctive. Hyphae hyaline, becoming pale brown, interwoven, thin-walled, septate, 1.5-3.5 μ in diameter. Conidiophores simple to occasionally branched, hyaline, thin-walled, septate, up to 30 x 3 μ with conidia borne in clusters at the tips, hyaline, elongate-ellipsoid to ovoid, 2.5-7.5 x 1-2.5 μ or "oval und 4.5-7.6 x 3.2-5 (Mittel 6.1 x 4.1) μ " (Mathiesen-Käärrik).

Illustrations: Mathiesen-Käärrik, Aino. 1953. Meddel. statens Skogsforskningsinst., Sweden, 43(4): 1-74, Figs. 4 and 5d.

C. albida was found on spruce and pine associated with bark beetles (1-2; culture JH-62 = J12-52, AM-K). No type of the species has been designated, but herbarium specimens and cultures are preserved at the Statens Skogsforskningsinstitut, Experimentalfältet, Sweden. This species causes little or no discoloration and is unimportant as a staining fungus.

No. 36, *C. minor*, differs from *C. albida* in the shorter necks and the larger crescent- to sickle-shaped ascospores. No. 39, *C. narcissi*, is similar but has broadly ellipsoid ascospores and occurs on narcissus bulbs.

38. *Ceratocystis minuta* (Siem.) Hunt, comb. nov. ✓

Ophiostoma minutum Siem., *Planta Polonica* 7(3): 23. 1939.

Perithecia superficial to immersed, the bases dark brown to black, 50-75 μ in diameter or "84-140 μ altis, 74-125 μ diam." (Siemaszko), unornamented but somewhat rough appearing; necks black, short, blunt, 50-150 μ long, 15-24 μ in diameter at the base and 7-15 μ at the tip; ostiolar hyphae pale brown at the base, hyaline at the tip, erect, pointed, 10-15 in number, up to 45 μ long and 2.5 μ in diameter at the base; asci not seen; ascospores filiform, pointed at each end, somewhat curved, 8-13 x 0.5-1.5 μ (Plate 1, Fig. 11), or "8-10 (12.5) x 1-1.5 μ " (Siemaszko).

Colonies appressed, with sparse aerial mycelium, white or becoming grayish; sparse conidia formed in concentric zones; perithecia occurring in sectors or in concentric zones; undersurface of the agar unchanged; odor "schwach rettigartig" (Mathiesen, 1951). Hyphae hyaline, thin-walled, septate, up to 3.5 μ in diameter. Conidiophores sparse, short, hyaline, up to 25 x 1.5 μ , with conidia hyaline, ellipsoid to ovate, 3-4 x 1.5 μ , formed acrogenously at the tips of the conidiophores.

Illustrations: Siemaszko, W. 1939. *Planta polonica* 7(3):1-52, Plate III, Figs. 10-12, and Fig. 1B; Davidson, R. W. 1942. *Mycologia* 34: 650-662, Fig. 3F-H, and Fig. 4A-C; Mathiesen, Aino. 1951. *Sv. Bot. Tidskr.* 45: 203-232, Fig. 1.

C. minuta was described from Poland on spruce infested with bark beetles (Siemaszko, 1939). No type material has been seen, nor is it known if a type collection has been designated. The species is known from Sweden (1-1, AM-K), and from the United States (BPI). It is not known to be a staining fungus.

The filiform ascospores of this species are unique in the genus *Ceratocystis*.

39. *Ceratocystis narcissi* (Limber) Hunt, comb. nov.*Ophiostoma narcissi* Limber, Phytopath. 40: 493. 1950.

Perithecia superficial to partially immersed, the bases black, globose, up to 200 μ in diameter, unornamented; necks black, pale brown to hyaline at the apex, often bent, up to 350 μ long, 20–25 μ in diameter at the base and 10–20 μ at the tip; ostiolar hyphae not seen, "ostiole usually entire, but very rarely the hyphal strands of the beak separate at the apex producing a fimbriate margin" (Limber); asci not seen; ascospores broadly ellipsoid, 2.5–3.5 \times 1.5–2 μ (Plate 1, Fig. 9) or "ovate to oblong with rounded ends, . . . 2–3.7 \times 2–2.5 μ " (Limber).

Cultures forming a dull white almost completely appressed mat at first, with scattered aerial hyphae; becoming yellowish with age; after 2–3 weeks abundant perithecia produced; undersurface of the agar unchanged to pale brown; growth intermediate, 20 mm.; odor not distinctive. Hyphae hyaline, interwoven, branched, thin-walled, septate, 1.5–3 μ in diameter. Conidiophores hyaline, thin-walled, septate, up to 30 \times 2 μ or "1–2 μ wide by 2–50 μ long, often tapering toward the apex to about 0.8 μ in diam." (Limber), with conidia hyaline, variable in size and shape, mostly ovate, often pointed at one end, 2.5–8 \times 1.5–3 μ , the conidia borne on short sterigmata-like projections at the tips of or along the sides of the conidiophores.

Illustrations: Limber, D. P. 1950. Phytopath. 40: 493–496, Figs. 1 and 2.

C. narcissi was described from narcissus bulbs imported into the United States from Holland, England, and Ireland (TYPE, BPI-A86579 Hob. 5332; culture JH-38 from CBS). The fungus is a saprophyte or a weak parasite, and is not a serious pathogen. When associated with other bulb pests it may penetrate deeply into the bulb and hasten its decay (Limber, 1950).

The broadly ellipsoid ascospores differentiate *C. narcissi* from the other species with short necks.

SPECIES IMPERFECTLY KNOWN

40. *CERATOSTOMELLA ACOMA* Miller & Cernzow, State Forestal Tech. Publ. Office, Moscow, 1934.

"Perithezien . . . rund 200–250 μ im Durchmesser mit schmalem über 1 mm. langem und 30–40 μ (unten) breitem, allmählich sich verengendem Hals. Wimpern an der Mündung des Halses fehlen gänzlich . . . Ascosporen cylindrisch 1.6 \times 5.2 μ . Konidienträger-*Graphium*-ähnlichen Koremien . . . mit kurzem, farblosen, cylindrischen oder umgekehrt kegelförmigem 100–300 μ langem Fuss. Konidien kurz stäbchenförmig 2.5–5.7 μ lang, 2–3 μ breit, von Schleim umgeben und einen farblosen Tropfen am Scheitel des Koremiums bildend.

"Erregt eine intensiv blauschwarze Färbung des Kiefernholzes. Fundort: Sosnowka, Provinz Nijnij-Nowgorod (Gorkij)" (Miller and Cernzow).

The publication data above are cited from R.A.M. 14: 271. 1935. No material of this species could be obtained. Based on the above description *C. acoma* appears to be similar to No. 21, *C. galeiformis*, which, however, differs in the hat-shaped ascospores.

41. CERATOSTOMELLA BUXI Boris, U.S.S.R. Central Forestry Res. Inst. Bul. 2: 7-42. 1934.

"Perithecia globosa, 300-350 μ diametro; pilis 100-300 μ longis, raris rufescentibus tecta. Rostrum 300-560 μ longum, 30-55 μ diam., eciliatum. Nonnulla perithecia rostris duobus instructa. Asci hyalini, clavati, sursum attenuati, 15-22.5 μ longi, 4.5-6 μ lati. Sporae hyalinae, cylindricae, parum curvatae, 3-6 μ longae, 0.75-1.5 μ latae.

"Hyphae of *Ceratostomella buxi* in boxwood are brown, segmented, with distinct walls, slightly branching, 4-6 μ in diameter."

The above description is taken from a translation on file at the former Division of Forest Pathology, Beltsville, Maryland. Material of *C. buxi* could not be obtained. Based on the above description the species appears to be similar to No. 11, *C. penicillata*, and No. 13, *C. serpens*. The above article is abstracted in R.A.M. 14: 62. 1935.

42. CERATOSTOMELLA CASTANEAE Vanin & Solov., Plant Protection Bul., Leningrad, 5: 122. 1932.

"Peritheciis ovalibus, superficialibus, 84-43 [x] 68-85 μ , basi pilis 200-357 μ longis dense tectis, rostello 1.1-1.8 mm. longo, apice setulis 14.7-20 μ longis numero 10 praedito. Ascosporis bacillaribus, hyalinis, 4.4-7.4 μ longis.

"In ligno, quod cinero-nigrum tingit, *Castaneae vescae*. Saepe, in distr. Sotschi regionis Maris Nigr. VIII-IX, 1929" (Vanin and Solovjev).

Material of this species could not be obtained. Based on the above description *C. castaneae* appears to be distinct from all other species of *Ceratocystis* in the combination of small perithecial bases, long necks, short ostiolar hyphae, and the rod-shaped ascospores. In perithecial features it seems to be like No. 31, *C. schrenkiana*, but differs in the bacilliform ascospores.

43. OPHIOSTOMA CATONIANUM Goid., R. Staz. Pat. Veg. Bol., Rome, n. s., 15: 125. 1935.

"Mycelio initio hyalino (hyphis 1.5-2 μ diam.), postea vero obscuriore et leniter ruguloso (hyphis 2.5 μ diam.); peritheciis superficialibus vel parum matrice immersis, subglobosis, carbonaceis, 110-165 μ diam., hyphis nigris tenuibus plerumque vestitis; ostiolo cylindraceo, recto vel leniter flexuoso, atro, 475-630 μ longo et basi, 32-42 μ , summo, 11-14 μ crasso, apicem versus minus obscuro ibique ciliis hyalinis coronato; ciliis numerosis subuliformibus, continuis 25-65 x 0.8-2.5 μ . Ascis cito diffluentibus in interna parte perithecorum irregulariter dispositis; ascosporis hyalinis, curvulis, poene allantoideis, mucro obvolutis, 3.5 x 1.5-1.8 μ , maturitate in cirro expulsi vel globulum lactiginosum albumcitrinum in apice ostioli efformantibus.

"Statis conidicis duobus ad genus *Graphium* Corda (*Graphium pirinum* G. Goid.) et *Hyalodendron* Diddens (*Hyalodendron pirinum* G. Goid.) referentibus.

"Hab. in ligno *Piri communis* L. in valle "di Non" et in valle "di Sole" Italiae; anno 1934" (Goidanich).

Goidanich states (letter) that all preserved material of this species

has been destroyed. A culture of *O. catonianum* from CBS is atypical and no longer of any value in species identification. *O. catonianum* appears to be similar to No. 22, *C. piceae*, and No. 26, *C. ulmi*, but these two species have shorter ostiolar hyphae. *C. ulmi* has, as well, larger ascospores and smaller perithecia than *O. catonianum*.

44. CERATOSTOMELLA COMATA Miller & Cernzow, State Forestal Tech. Publ. Office, Moscow. 1934.

"Peritheciis . . . klein, rund 90–122 μ im Durchmesser, seltener h  hen als breit. Hals 255–480 μ lang, unten 26–33 μ , am Gipfel 20–26 μ breit. An der M  ndung des Halses ein Kranz aus 14–24 ungleichen dunklen 35–130 μ langen, unten 3 μ , am Ende 1.5 μ breiten Wimpern, die ohne scharfe Grenze in die Hyphen des Halses   bergehen. Ascosporen 3.6 x 6 μ , schwach gebogen. Konidientr  ger-*Graphium*-  hnliche, garbenf  rmige 150–200 μ hohe Koremien . . . Konidien 3 x 6.5 μ von Schleim umgeben, einen gr  nlich braunen Tropfen am Scheit  tel des Koremiums bilden. Oft besteht der Fuss der Koremien im unteren Teile nur aus einer einreihiger Hyphe . . . Solche Konidientr  ger bilden einen   bergang zwischen dem *Graphium* und *Haplographium*-typus.

"Erregt eine graugr  nliche F  rbung des Kiefernholzes. Fundort: Sosnowka, Provinz Nienij Nowgorod (Gorkij)." (Miller and Cernzow).

The publication data for this species are cited from R.A.M. 14: 271. 1935. Material of this species could not be obtained. *C. comata* appears to be similar to No. 17, *Ceratocystis olivacea*, No. 18, *C. obscura*, and No. 16, *C. leptographioides*, based on perithecial morphology, but apparently differs from these in combinations of the perithecial dimensions, the size of the ascospores, and in the imperfect stages.

45. CERATOSTOMELLA FAGI Loos, Arch. f. Mikrobiol. 3: 376. 1932.

"1. Fungus ascophorus: Peritheciis plus minus gregariis, partim subsuperficialibus, carbonaceis, aterrimis, globosis, 135–220 μ diametr., longe (860–1920 μ) rostratis, ostiolo ciliis 12–58 μ longis, 1–2 μ crassis circumdato; sporidiis ellipsoideis, interdum paulum curvatis, 2.5–3.5–4.7 μ longis, 0.7–1.0–1.2 μ latis, globulo terminali expulsis.

"2. Fungus conidiophorus: a) Mycelio hyalino, dein griseo, conidiis modo *Cladosporii* et *Cephalosporii* fictis, plerumque biguttulatis, 6–15 μ longis, 2.5–4 μ latis; b) *Graphium* coremiis gregariis, 420–510–625 μ altis, 8–11–23 μ crassis, stipite atro, conidiophoris hyalinis, conidiis cylindricis, 3–5 μ longis, 1.2–2.2 μ latis, capitulum cum gutta mucosa formantibus; forsan *Graphii penicilloidis* Corda varietas.

"Habit. in ligno *Fagi silvaticae* L." (Loos).

A culture of *C. fagi* (JH-39 from CBS) is now atypical and of no value for identification of the species. No perithecial material could be obtained. *C. fagi* appears to differ from No. 22, *Ceratocystis piceae*, in that the latter has shorter thicker ostiolar hyphae and wider ascospores. Further work may show that *C. fagi* is a synonym of *C. piceae*.

46. CERATOSTOMELLA IMPERFECTA Miller & Cernzow, State Forestal Tech. Publ. Office, Moscow, 1934.

"Perithechien rund 156–208 μ im Durchmesser mit etwa zweimal so langem und 39–45 μ breiten Hals . . . Öffnung des Halses konnte niemals beobachtet werden. Ascosporen cylindrisch 2.6–2.8 x 5–5.5 μ . Konidienträger von *Haplographium* typus . . . , schwarzbraun 150–600 μ hoch, 6–7 μ dick, oben zwei-dreimal pinselig büschelförmig verzweigt, Endzweige mit zahlreichen farblosen Sterigmen. Konidien von Schleim umgeben, einen hutartigen weissen Ko[p]f am Scheitel des Konidienträgers bildend. Die Konidienträger von *C. imperfecta* sind *Haplographium bicolor* Grove, der auch von Schleim umgebene und zu einem apicalem Köpfchen verklebte Konidien bildet, verwandt.

"Erregt eine intensive grauschwarze Färbung des Kiefern und Fichtenholzes.

"Fundort: Umgebungen von Moskau, Stalingrad, Provinz Untere Wolga." (Miller und Cernzow).

The publication data for this species are cited from R.A.M. 14: 271. 1935. No material could be obtained for study. On the basis of the above description, *C. imperfecta* appears to be a synonym of No. 11, *Ceratocystis penicillata*, as no apparent differences are noted in the perithecia and the ascospores. The *Halographium* stage of *C. imperfecta* appears to be very similar to the *Leptographium* stage of *C. penicillata* and it has been suggested that those species of *Haplographium* with hyaline conidia might be placed in *Leptographium* (Shaw and Hubert, 1952).

47. CERATOSTOMELLA LIGNORUM Wollernw. & Stapp, Biol. Reichsanst. f. Land u. Forstw. Arb., Berlin, 16: 310–312. 1928.

Perithecia emergent through the mycelial mat, the bases 140–300 μ in diameter, ornamented with dark-colored radiating hyphae; necks about 500–2000 μ long and 10–30 μ in diameter; ostiolar hyphae spreading, pointed, about 15 in number, up to 30 μ long; asci evanescent, only indistinctly seen; ascospores cylindrical, slightly curved, 4.4 x 1.2 μ , varying from 3–7 x 1–2 μ , and extruded from the tip of the neck in a spore tendril or collecting in a whitish drop.

Cultures at first with a whitish aerial mycelium of loosely interwoven hyphae, the substrate soon turning black; perithecia appearing after many weeks, breaking through the mycelial mat. Substrate hyphae composed of thick, dark-colored hyphae. Conidiophores of two types, the one of short side branches, often occurring in pairs, up to 20 μ long, conidia produced at the tips ellipsoid, cylindrical or comma-shaped, 5.4 x 1.4 μ , usually 5.3–5.5 x 1.1–1.7 μ , greatest range 4–7 x 1–2 μ ; the other thickened upper portions of branches with numerous sterigmata on all sides and with conidia as above.

In pure culture it appears macroscopically like a *Graphium*, but microscopically, instead of coremia, it has conidiophores of another type and perithecia with a structure as in *Ceratostomella*.

Found on decayed spruce wood.

The above description is adapted from the original description of the species as no material of *C. lignorum* could be obtained. The species appears to be similar to No. 33, *C. tetropii*, in most of the perithecial characters but *C. tetropii* has longer ostiolar hyphae and shorter ascospores.

48. CERATOSTOMELLA MEROLINENSE Geörgew., Mitt. Inst. Forstwissenschaft. Forsch., Belgrade, 16: 25-29. 1930.

Perithecia 160-320 μ tall, the bases 28-32 μ in diameter, necks 20 μ thick; ostiolar hyphae 28 x 4 μ ; asci cylindric, 80 x 4 μ , in the lower thickened portion of the perithecium; ascospores cylindrical, rounded at both ends, with numerous metachromatic droplets, 8-10 x 4 μ .

Hyphae hyaline, branched at right angles, 2-3 μ in diameter. Conidiophores are coremia of the *Graphium* type, brown at the base, light colored at the tip, 80-160 μ long and 16-20 μ wide, with conidia cylindrical and somewhat bent, 4 x 2 μ ; mycelial conidia produced directly on the vegetative hyphae, 10 x 4 μ .

Described from oak in Yugoslavia.

The above description is adapted from the German summary in the paper cited. Material of this species could not be located. *C. merolinense* appears to be different from other species of *Ceratocystis* in the very small base of the perithecium. The long cylindrical asci suggest that it may belong in a different genus.

49. CERATOSTOMELLA MICROCARPA Karsten, Hedwigia 17: 86. 1884.

"Perithecia gregaria, erumpentia, denique libera, vulgo sphaeroidea, atra, latit. circiter 0.3 mm, rostro cylindraceo, aequali, laevi, perithecium aequante vel eo duplo longiore. Asci crasse clavati, longit. 18-25 mmm, crassit. 4 mmm. Sporae conglobatae s. tristichae, elongatae vel cylindraceae, curvulae, simplices, hyalinae, longit. circiter 4 mmm, crassit. 0.5-1 mmm.

"In ligno vetusto *Populi tremulae* prope Mustiala, m. Majo.

"A congeneribus minutie partium omnium statim tuteque diagnosticur." (Karsten).

No material of this species was examined. On the basis of the above description *C. microcarpa* cannot be placed with any of the known species of *Ceratocystis*. The species appears to belong in a separate genus on account of the persistent asci.

50. CERATOSTOMELLA STENOCERAS Robak, Nyt Mag. Naturvidensk., Oslo, 71: 214. 1932.

"Hyphae hyalinae, longicellulares, 1-2 μ crasse. Perithecia carbonacea, atra, globosa, 130-250 μ diam. interdum pyriformia, hyphis numerosissimis, atrobrunneis, c. 2 μ crassis, ad 125 μ longis dense ornata. Rostellum laetius, glabrum, subcylindricum, apicem versus paullo angustatum, 120-150 μ longum, ad basem circ. 20 μ crassum, apice 8-12 μ crassum et ciliis coronatum. Cilia 10-20, hyalina, continua, 20-48 (60) μ longa, subulato angustata, apice saepe obtusa, ad basem 2.6-2.7 μ , apice c. 0.5 μ crassa. Cilia subterminalia interdum evoluta. As[c]i non visi. Sporidia hyalina, continua, ceterum ut in *C. pluriannulata* sens. Lagerberg et Melin, sed minora, 2-2.9 μ longa, 1.3-1.4 μ lata.

"Formationem perithecorum formatio conidiorum praecedit. Conidia numerosissima, hyalina, continua, elongata v. ellipsoidea v. ovata, 3.4-6.9 μ longa, 2-3.4 μ lata, e conidiophoris vel interdum e hyphis

ipsis formata. Conidiophori e type Cylindrocephali-cephalosporii vel Cladosporii. Conidia separata interdum gemmipara.

"Habitat in pulpa artificiali ligni coniferarum. In substrato solido (agar-agar) solutione fermenti decocto cultivata et observata." (Robak).

A culture of this species (JH-1 from CBS) is now atypical and of no value for species identification. Perithecial material of *C. stenoceras* has not been obtained. It appears to be similar to No. 27, *C. plurianulata*, which differs in the larger ascospores.

EXCLUDED SPECIES

The following two species have persistent asci with allantoid ascospores which are not exuded in a sticky drop at the tip of the neck:

CERATOSTOMELLA MICROSPORA Ell. & Ev., Acad. Nat. Sci. Phil. Proc. **45**: 444. 1893, not *Ceratostomella* (*Ophiostoma*) *microspora* Davids. (1942), (TYPE, FH; BPI, NY).

CERATOSTOMELLA ECHINELLA Ell. & Ev., No. Amer. Pyreno., p. 185. 1892, (Authentic, BPI, FH).

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acoma, Ceratostomella, p. 50
 adiposa, C. p. 10
 albida, C. p. 48
 ambrosia, C. p. 44
 autographa, C. p. 23

brunneo-ciliata, C. p. 32
 bunae, Endoconidiophora, p. 18
 buxi, Ceratostomella, p. 51

cana, C. p. 35
 capillifera, C., p. 42
 castaneae, Ceratostomella, p. 51
 catonianum, Ophiostoma, p. 51
 clavata, C. p. 37
 coerulea, Ceratostomella, p. 41
 coerulescens, C., p. 21
 coffeae, Rostrella, p. 14
 comata, Ceratostomella, p. 52

echinella, Ceratostomella, p. 41, 55
 exigua, ", p. 47

fagacearum, C., p. 21
 fagi, Ceratostomella, p. 52
 fimbriata, C., p. 14
 floccosa, C., p. 36

galeiformis, C., p. 35

imperfecta, Ceratostomella, p. 53
 ips, C., p. 30

leptographoides, C. p. 28
 lignorum, Ceratostomella, p. 53
 longirostellata, C. p. 42

major, C. p. 12
 merolinense, Ceratostomella p. 54
 microcarpa, " p. 54
 microspora, " p. 46
 microsporum, Ophiostoma, p. 46
 minor, C., p. 47
 minus, Ophiostoma
 minuta, C. p. 49

moniliiformis, C., p. 18
montia, C. p. 45
multiannulata, D. p. 40

narcissi, C. p. 50

obscura, C. p. 30-
olivacea, C. p. 29

paradoxa, C. p. 19
penicillata, C. p. 24
perparvispora, C. p. 46
piceae, C. p. 34
piceasperda, C. p. 25
pilifera, C. p. 41
pini, Ceratostomella p. 47
pluriannulata, C. p. 39
polonicum, C. p. 27
pseudotsugae, Ceratostomella, p. 47

querci, Ceratostomella, p. 34

radidicola, C. p. 20
rostrocyllindrica, C. p. 26

schrenkiana, C. p. 43
serpens, C. p. 25
stenoceras, Ceratostomella, p. 54

tetropii, C. p. 45

ulmi, C., p. 38

variospora, C., p. 38
virescens, Endoconidiophora, p. 21

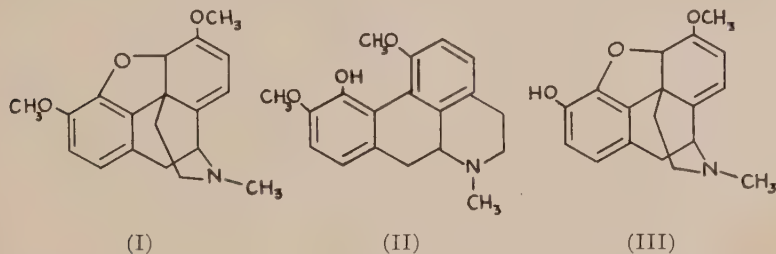
wilsoni, C. p. 18

Alkaloids of *Papaver Orientale* L. I. Qualitative Detection and Occurrence¹

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J. Gadamer (2) and W. Klee (3) long ago reported the isolation from *Papaver orientale* L. of the minor opium alkaloid thebaine (I), of a new alkaloid isothebaine (II) to which was assigned a morphothebaine-type ring structure, and of two lesser alkaloids which they believed to be identical with glaucidine and protopine. Klee mentioned the possible biosynthetic significance of the occurrence of both morphine and morphothebaine ring systems in the alkaloids of the same species and gave some substance to his suggestion by reporting opposing seasonal trends in tissue concentrations of thebaine and isothebaine, respectively.



Konovalova, Yunusov and Orekhov (4) obtained thebaine but not isothebaine from wild plants collected in the summer in the Transcaucasus. They reported the presence of a new alkaloid oripavine (III) to which they assigned ultimately (5) the structure 3-O-demethylthebaine. Fulton (1) records his inability to detect thebaine in a sample of Oriental poppy capsules of presumably domestic horticultural origin. Isothebaine was present. A minor alkaloid presumed to be glaucidine was obtained as well as several alkaloids which were not so readily characterized.

With the objectives of clarifying present uncertainties of distribution and of providing a basis for future studies of alkaloid biosynthesis within this species, we have undertaken an investigation of the major alkaloids of the Oriental poppy.

METHODS

A paper chromatographic method was sought for separation of

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thebaine from isothebaine in very small amounts. Many organic solvents customarily employed for chromatography of alkaloids were tested both with and without the addition of buffers, but all failed to effect the desired separation. As an alternative approach, known differences in the water solubility of thebaine and isothebaine salicylates and tartrates were successfully exploited. A description of the procedures is given below.

Spots of alkaloid solutions or extracts are applied to paper sheets which previously have been impregnated either with saturated aqueous salicylic acid solution or with 1 per cent (w/v) aqueous tartaric acid (rac.) solution and dried. The sheets are then placed in a chromatographic chamber of the ascending or descending type containing the same organic acid solution as was employed for impregnating the paper. When the solvent front reaches the desired height the sheets are removed, dried by hanging in the air and sprayed with Munier's modification (6) of Dragendorff's reagent or with dilute nitrous acid solution. With the former reagent, both thebaine and isothebaine yield orange-red spots: with nitrous acid isothebaine, but not thebaine, yields an intense violet color. Both the linear dimensions of the spots and the intensity of the colors produced are proportional to the amount of alkaloid added to the paper.

Pure thebaine usually migrates with an R_f value of 0.70 in the salicylic acid solvent and 0.80 in tartaric acid. Inequalities in distribution of the impregnating substances in the paper sheet, the presence of impurities in plant extracts, and fluctuations in ambient temperature may alter these figures markedly. Pure isothebaine, obtained through the kindness of Dr. E. Schlittler, usually migrates with an R_f value of approximately 0.41 in the salicylic acid solvent and 0.56 in tartaric acid. These figures are also subject to fluctuation for the same reasons listed above. Fortunately, the R_f values for both alkaloids usually increase or decrease together, depending upon the cause of the variation, so that effectiveness of separation is not impaired. Previous impregnation of the paper sheets with the same solvent to be used in running the chromatograms, while not essential to successful separation of the two alkaloids, appeared to yield more consistent results under our conditions.

Extracts of dry plant material suitable for chromatography can be made quite simply. One g. of dried and powdered material is shaken for 1 hr. in a bottle containing 1 ml. conc. ammonium hydroxide and 20 ml. of ethyl ether and closed tightly with a plastic-lined screw cap. At the end of the extraction, 15 ml. of ether are withdrawn by pipette and the ether removed in vacuo. The residue from the evaporation is taken up in a few drops of ethanol and can be used directly for application to the paper sheets. Impurities causing variation in R_f values can usually be eliminated by the following additional step.

Fifteen ml. of the original ether extract are pipetted directly into a separatory funnel containing 10 ml. of 1 per cent citric acid in water (w/v). This is shaken and the acid layer drawn off. A second portion of acid is added and the procedure repeated. The combined acid extracts are made alkaline with 10 per cent (w/v) sodium carbonate solution and extracted twice with 20 ml. portions of ethyl ether. The

combined ether extracts are then reduced to dryness as above and the residues taken up in ethanol. By this means, about 95 per cent of the thebaine and 65 per cent of the isothebaine originally present in the sample are recovered.

All plant tissues employed in the above procedures were dried either by exposure to sun and air in the open greenhouse in June or by forced-draft air at 80° C. in a specially constructed drying oven. The consequences of such treatment appear to have some bearing upon the patterns of alkaloidal components and will be discussed in a succeeding paper.

Owing to the mode of vegetative proliferation of these plants from the seedling crown, it is possible to remove from older specimens a complete botanical unit consisting of roots, leaves and flower stalk without injury to the parent plant and without encountering serious errors in proportional representation of the different organs in the samples intended for assay.

A modification of the above chromatographic techniques was found useful for application to individual plants growing in the field without necessity of prior collection of tissue samples. Disks of filter paper (11 cm. diameter) are cut with scissors in such a manner that a short projection approximately 3 cm. long and 3-4 mm. wide emerges from the center of and at right angles to the surface of the disk. The disks are then impregnated with a saturated aqueous solution of salicylic acid and dried in the air. A single drop of latex, collected from the freshly cut petiole of a leaf, is applied to the center of the disk in such a way that it does not completely block the area of confluence of projection with rest of disk. The disk is then placed over the edges of a Petri dish bottom or lid (9 cm. diameter) with the projection dipping as a wick into the salicylic acid solution contained in the dish. Another dish of equal diameter is inverted over the assembly to hold the paper in position and to retain water vapor. When the solvent front advances to the rim of the dish the disk is removed and hung to dry. Alkaloid zones are developed by spraying with Draggendorf's reagent. Large numbers of chromatograms can be run concurrently in this fashion.

RESULTS

Spots corresponding in position on the chromatograms to thebaine and to isothebaine were readily obtained on pooled samples representing the dried, powdered leaf, flower stalk and root of many plants grown from domestically obtained seed.

Spectrophotometric identification of the component of the spot corresponding with isothebaine in R_f value was easily attained by elution of several such spots and by purification of the eluted alkaloid by one cycle of acid-ether extraction. Identification of the spot corresponding to thebaine was difficult owing to the smaller quantities present and to background absorption. However, by utilizing larger amounts of plant extract applied to specially washed columns of powdered filter paper, we have been able to obtain spectrophotometrically pure thebaine. This work will be described in a succeeding paper inasmuch as it relates

to the development of a quantitative method of separation and of assay of the major alkaloids of the species.

When the ascending or descending chromatographic technique was applied to extracts of plants grown from domestic seed, a wide range of variation was observed. These plants were grown in the greenhouse through the winter of 1951-1952 as seedlings and were set in a specially prepared field plot in May, 1952. Individual plant samples were taken in June, 1953. In a total populational sample of 297 plants, 102 contained detectable amounts of both thebaine and isothebaine; 192 contained only isothebaine; and none contained only thebaine. Three plants contained no alkaloids within the limits of sensitivity of the method. When thebaine was present, the isothebaine spot was always the larger and more intensely colored of the two. Samples taken from the same plants at other times of the year, whether grown in the field or in the greenhouse, exhibited seasonal fluctuations in apparent alkaloid content with the largest and darkest spots associated with samples harvested in June (young capsule stage) coincident with the highest latex content. Although no indication of opposing seasonal trends in isothebaine and thebaine concentrations could be detected by this method, quantitative analytical data will be required to assess the observations reported by Klee (3).

Chromatograms of extracts taken from individual plants of a given clonally propagated (root cuttings) population invariably bore remarkably uniform patterns of alkaloid spots with respect to number, color intensity and size. The probability of clonal stability is thus indicated.

By the method of circular paper chromatography the population of seedling plants described above was again sampled in the late summer and early autumn of 1954. In a total of 468 individuals only 1 contained no detectable thebaine. None failed to contain isothebaine. The latter was again the dominant alkaloid. It is very likely that the direct use of latex for paper chromatograms may provide greater sensitivity, especially for the detection of thebaine, but the possibility of encountering thebaine losses during the drying of poppy tissues cannot at this time be discounted.

A third alkaloid appeared on circular chromatograms of extracts of two plants out of the seedling population of 468 examined. The R_f value for this alkaloid was approximately 0.23. The possibility that it may be oripavine has led us to propagate one of the plants by vegetative means for further examination.

One group of plants obtained originally from a single commercial source was propagated clonally and grown during the winter of 1953-1954 in the greenhouse. By the end of January, 1954, the plants had begun to drop leaves owing to excessive root binding in the clay pots. It is of interest to note that, under these circumstances but not under more favorable conditions of growth, these plants yielded ascending-type chromatograms which contained uniformly four alkaloids of R_f 0.08, 0.42, 0.72 and 0.88. The second and third in this series are isothebaine and thebaine, respectively. The fourth yielded a pink color rather than orange-red with Draggendorf's reagent and, when eluted

from the chromatograms and purified by a single solvent extraction cycle, possessed a strong absorption band in the ultraviolet at $260\text{ m}\mu$ and a smaller maximum at about $285\text{ m}\mu$. Its identity is unknown. Indications of the presence of the alkaloid with lowest R_f value in a substantial number of other plants of different origin have been obtained.

Figure 1 depicts one of the chromatograms bearing the four alkaloidal spots described above together with a scanning diagram obtained by measuring the optical density of the paper strip at regular intervals along its length in a reel adapter for the Beckman model DU spectrophotometer at $540\text{ m}\mu$ wavelength.

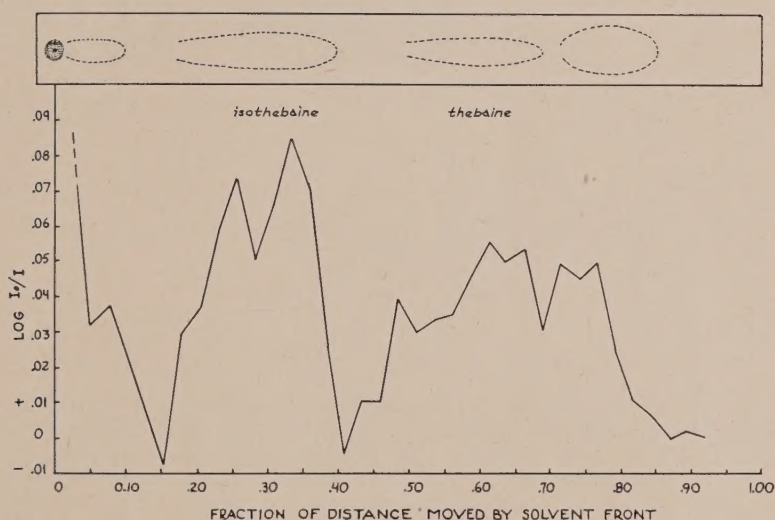


FIG. 1. Coincidence between spectrophotometric scanning data at wavelength $540\text{ m}\mu$ and location of visible spots on chromatogram of clone R-3.

SUMMARY

One-dimensional linear and circular paper chromatographic methods for the separation and detection of the alkaloids thebaine and isothebaine in less than single plant samples of *Papaver orientale* L. are described. These methods were applied to seedling and to clonally propagated populations, and qualitative variations in alkaloid distribution are reported. Isothebaine appeared to be the major alkaloid in all cases. At least two other alkaloids of unknown identity were detected on the chromatograms of some plants.

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